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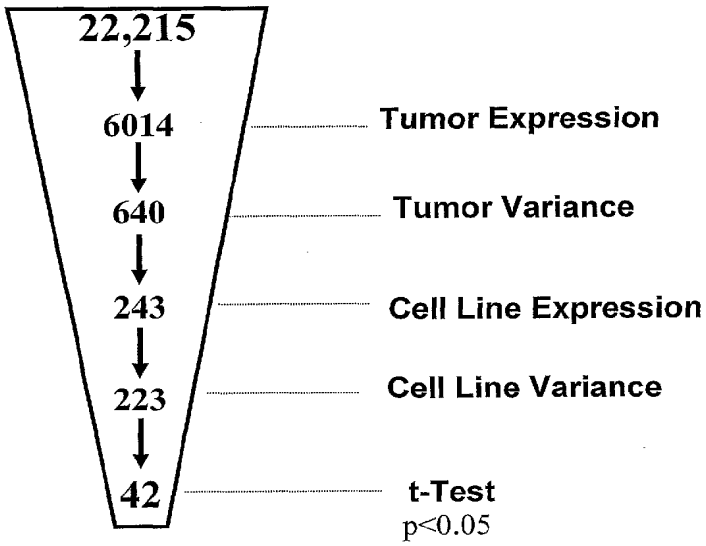

(57) Abstract: EGFR biomarkers useful in a method for predicting the likelihood that a mammal that will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises (a) measuring in the mammal the level of at least one biomarker selected from epiregulin and amphiregulin, (b) exposing a biological sample from the mammal to the EGFR modulator, and (c) following the exposing of step (b), measuring in the biological sample the level of the at least one biomarker, wherein an increase in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates an increased likelihood that the mammal will respond therapeutically to the method of treating cancer.

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BIOMARKERS AND METHODS FOR DETERMINING SENSITIVITY TO EPIDERMAL GROWTH FACTOR RECEPTOR MODULATORS

SEQUENCE LISTING:

A compact disc labeled "Copy 1" contains the Sequence Listing as 10646 PCT.ST25.txt. The Sequence Listing is 1241 KB in size and was recorded August 24, 2006. The compact disk is 1 of 2 compact disks. A duplicate copy of the compact disc is labeled "Copy 2" and is 2 of 2 compact discs.

The compact disc and duplicate copy are identical and are hereby incorporated by reference into the present application.

FIELD OF THE INVENTION:

The present invention relates generally to the field of pharmacogenomics, and more specifically to methods and procedures to determine drug sensitivity in patients to allow the identification of individualized genetic profiles which will aid in treating diseases and disorders.

BACKGROUND OF THE INVENTION:

Cancer is a disease with extensive histoclinical heterogeneity. Although conventional histological and clinical features have been correlated to prognosis, the same apparent prognostic type of tumors varies widely in its responsiveness to therapy and consequent survival of the patient.

New prognostic and predictive markers, which would facilitate an individualization of therapy for each patient, are needed to accurately predict patient response to treatments, such as small molecule or biological molecule drugs, in the clinic. The problem may be solved by the identification of new parameters that could better predict the patient's sensitivity to treatment. The classification of patient samples is a crucial aspect of cancer diagnosis and treatment. The association of a patient's response to a treatment with molecular and genetic markers can open up new opportunities for treatment development in non-responding patients, or distinguish a treatment's indication among other treatment choices because of higher confidence in the efficacy. Further, the pre-selection of patients who are likely to respond well to a medicine, drug, or combination therapy may reduce the number of patients needed in

a clinical study or accelerate the time needed to complete a clinical development program (Cockett et al., Current Opinion in Biotechnology, 11:602-609 (2000)).

The ability to predict drug sensitivity in patients is particularly challenging because drug responses reflect not only properties intrinsic to the target cells, but also a host's metabolic properties. Efforts to use genetic information to predict drug sensitivity have primarily focused on individual genes that have broad effects, such as the multidrug resistance genes, mdr1 and mrp1 (Sonneveld, J. Intern. Med., 247:521-534 (2000)).

The development of microarray technologies for large scale characterization of gene mRNA expression pattern has made it possible to systematically search for molecular markers and to categorize cancers into distinct subgroups not evident by traditional histopathological methods (Khan et al., Cancer Res., 58:5009-5013 (1998); Alizadeh et al., Nature, 403:503-511 (2000); Bittner et al., Nature, 406:536-540 (2000); Khan et al., Nature Medicine, 7(6):673-679 (2001); and Golub et al., Science, 286:531-537 (1999); Alon et al., P. N. A. S. USA, 96:6745-6750 (1999)). Such technologies and molecular tools have made it possible to monitor the expression level of a large number of transcripts within a cell population at any given time (see, e.g., Schena et al., Science, 270:467-470 (1995); Lockhart et al., Nature Biotechnology, 14:1675-1680 (1996); Blanchard et al., Nature Biotechnology, 14:1649 (1996); U.S. Patent No. 5,569,588).

Recent studies demonstrate that gene expression information generated by microarray analysis of human tumors can predict clinical outcome (van't Veer et al., Nature, 415:530-536 (2002); Sorlie et al., P. N. A. S. USA, 98:10869-10874 (2001); M. Shipp et al., Nature Medicine, 8(1):68-74 (2002): Glinsky et al., The Journal of Clin. Invest., 113(6):913-923 (2004)). These findings bring hope that cancer treatment will be vastly improved by better predicting the response of individual tumors to therapy.

The epidermal growth factor receptor (EGFR) and its downstream signaling effectors, notably members of the Ras/Raf/MAP kinase pathway, play an important role in both normal and malignant epithelial cell biology (Normanno et al., Gene 366, 2-16 (2006)) and have therefore become established targets for therapeutic development. Whereas the anti-EGFR antibody cetuximab and the EGFR small

molecular tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib have demonstrated activity in a subset of patients (Baselga and Arteaga, J. Clin. Oncol. 23, 2445-2459 (2005)), their initial clinical development has not benefited from an accompanying strategy for identifying the patient populations that would most likely derive benefit. The hypothesis that only a relatively small number of tumors are "EGFR-pathway dependent" and therefore likely to respond to EGFR inhibitors might explain the limited clinical activity that is observed with this class of therapeutics. For example, in patients with refractory metastatic colorectal cancer clinical response rates with cetuximab consistently range from 11% in a monotherapy setting to 23% in a combination setting with chemotherapy (Cunningham et al., N. Engl. J. Med 351, 337-345 (2004)). To date, significant efforts have been focused on elucidating the mechanisms of sensitivity or resistance to EGFR inhibition, particularly through evaluation of EGFR protein expression, kinase domain mutations, and gene copy number.

While relative protein expression of the EGFR as measured by immunohistochemistry (IHC) has been demonstrated in many solid tumors (Ciardiello and Tortora, Eur. J. Cancer 39, 1348-1354 (2003)), no consistent association between EGFR expression and response to EGFR inhibitors has been established. Clinical studies of cetuximab in a monotherapy setting and in combination with irinotecan in patients with mCRC failed to reveal an association between radiographic response and EGFR protein expression as measured by IHC (Cunningham et al., N. Engl. J. Med 351, 337-345 (2004); Saltz et al., J. Clin. Oncol. 22, 1201-1208 (2004)). Furthermore, clinical responses have been demonstrated in patients with undetectable EGFR protein expression (Chung et al., J. Clin. Oncol., 23, 1803-1810 (2005); Lenz et al., Activity of cetuximab in patients with colorectal cancer refractory to both irinotecan and oxaliplatin. Paper presented at: 2004 ASCO Annual Meeting Proceedings; Saltz, Clin Colorectal Cancer, 5 Suppl. 2, S98-100 (2005)). In comparison, clinical studies of erlotinib in NSCLC patients and gefitinib in ovarian cancer did demonstrate an association between EGFR expression, response, and survival (Schilder et al., Clin. Cancer Res., 11, 5539-5548 (2005); Tsao et al., N. Engl. J. Med., 353, 133-144 (2005)). The presence of somatic mutations in the tyrosine kinase domain, particularly in NSCLC has been extensively described (Janne

et al., J. Clin. Oncol., 23, 3227-3234 (2005)). In both preclinical and clinical settings, these mutations are found to correlate with sensitivity to gefitinib and erlotinib but not to cetuximab (Janne et al., J. Clin. Oncol., 23, 3227-3234 (2005); Tsuchihashi et al., N. Engl. J. Med., 353, 208-209 (2005)). In addition, the lack of EGFR kinase domain mutations in CRC patients suggests that such mutations do not underlie the response to cetuximab. EGFR gene copy number has also been evaluated as a potential predictor of response to EGFR inhibitors. Clinical studies of gefitinib demonstrated an association between increased EGFR copy number, mutational status, and clinical response (Cappuzzo et al., J. Natl. Cancer Inst., 97, 643-655 (2005)). A similar association was identified in a small number of patients treated with the anti-EGFR monoclonal antibodies cetuximab and panitumumab (Moroni et al., Lancet Oncol., 6, 279-286 (2005)). Additional potential predictive biomarkers have also been evaluated. For example, in glioblastoma patients, a significant association between co-expression of EGFRvIII and PTEN and response to EGFR small molecule inhibitors was found (Mellinghoff et al., N. Engl. J. Med., 353, 2012-2024 (2005)).

The anti-tumor activity of cetuximab has been attributed to its ability to block EGFR ligand binding and ligand-dependent EGFR activation. Clinical activity of cetuximab has been shown in multiple epithelial tumor types (Bonner et al., N. Engl. J. Med., 354, 567-578 (2006); Cunningham et al., N. Engl. J. Med., 351, 337-345 (2004)), however responses continue to be seen in only a fraction of patients. Previous attempts to identify predictors of sensitivity or resistance as described above have focused on specific biomarkers rather than using genomic discovery approaches. In addition, RNA-, DNA- and protein-based markers have rarely been examined in the same patient population in a single study, making comparisons challenging.

Biomarkers useful for determining sensitivity to EGFR modulators have been described in published PCT applications WO2004/063709, WO2005/067667, and WO2005/094332.

Needed are new and alternative methods and procedures to determine drug sensitivity in patients to allow the development of individualized genetic profiles which are necessary to treat diseases and disorders based on patient response at a molecular level.

SUMMARY OF THE INVENTION:

The invention provides methods and procedures for determining patient sensitivity to one or more Epidermal Growth Factor Receptor (EGFR) modulators. The invention also provides methods of determining or predicting whether an individual requiring therapy for a disease state such as cancer will or will not respond to treatment, prior to administration of the treatment, wherein the treatment comprises administration of one or more EGFR modulators. The one or more EGFR modulators are compounds that can be selected from, for example, one or more EGFR-specific ligands, one or more small molecule EGFR inhibitors, or one or more EGFR binding monoclonal antibodies.

In one aspect, the invention provides a method for predicting the likelihood a mammal will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker selected from epiregulin and amphiregulin; (b) exposing a biological sample from the mammal to the EGFR modulator; (c) following the exposing of step (b), measuring in the biological sample the level of the at least one biomarker, wherein an increase in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates an increased likelihood that the mammal will respond therapeutically to the method of treating cancer. In one aspect, the at least one biomarker comprises epiregulin and amphiregulin. In yet another aspect, the at least one biomarker further comprises at least one additional biomarker selected from Table 1. In another aspect, the biological sample is a tissue sample comprising cancer cells and the method further comprises the step of determining whether the cancer cells have the presence of a mutated K-RAS, wherein detection of a mutated K-RAS indicates a decreased likelihood that that the mammal will respond therapeutically to the method of treating cancer.

The biological sample can be, for example, a tissue sample comprising cancer cells and the tissue is fixed, paraffin-embedded, fresh, or frozen.

In another aspect, the EGFR modulator is cetuximab and the cancer is colorectal cancer.

In another aspect, the invention is a method for predicting the likelihood a mammal will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker that comprises CD73; (b) exposing a biological sample from the mammal to the EGFR modulator; (c) following the exposing of step (b), measuring in the biological sample the level of the at least one biomarker, wherein an increase in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates a decreased likelihood that the mammal will respond therapeutically to the method of treating cancer. In another aspect, the at least one biomarker further comprises at least one additional biomarker selected from Table 1. In another aspect, the method further comprises the step of determining whether the cancer cells have the presence of a mutated K-RAS, wherein detection of a mutated K-RAS indicates a decreased likelihood that that the mammal will respond therapeutically to the method of treating cancer.

A difference in the level of the biomarker that is sufficient to predict the likelihood that the mammal will or will not respond therapeutically to the method of treating cancer can be readily determined by one of skill in the art using known techniques. The increase or decrease in the level of the biomarker can be correlated to determine whether the difference is sufficient to predict the likelihood that a mammal will respond therapeutically. The difference in the level of the biomarker that is sufficient can, in one aspect, be predetermined prior to predicting the likelihood that the mammal will respond therapeutically to the treatment. In one aspect, the difference in the level of the biomarker is a difference in the mRNA level (measured, for example, by RT-PCR or a microarray), such as at least a two-fold difference, at least a three-fold difference, or at least a four-fold difference in the level of expression. In another aspect, the difference in the level of the biomarker is determined by IHC. In another aspect, the difference in the level of the biomarker refers to a p-value of <0.05 in Anova (t test) analysis. In yet another aspect, the difference is determined in an ELISA assay.

As used herein, respond therapeutically refers to the alleviation or abrogation of the cancer. This means that the life expectancy of an individual affected with the

cancer will be increased or that one or more of the symptoms of the cancer will be reduced or ameliorated. The term encompasses a reduction in cancerous cell growth or tumor volume. Whether a mammal responds therapeutically can be measured by many methods well known in the art, such as PET imaging.

The mammal can be, for example, a human, rat, mouse, dog, rabbit, pig sheep, cow, horse, cat, primate, or monkey.

The method of the invention can be, for example, an *in vitro* method wherein the step of measuring in the mammal the level of at least one biomarker comprises taking a biological sample from the mammal and then measuring the level of the biomarker(s) in the biological sample. The biological sample can comprise, for example, at least one of serum, whole fresh blood, peripheral blood mononuclear cells, frozen whole blood, fresh plasma, frozen plasma, urine, saliva, skin, hair follicle, bone marrow, or tumor tissue.

The level of the at least one biomarker can be, for example, the level of protein and/or mRNA transcript of the biomarker. The level of the biomarker can be determined, for example, by RT-PCR or another PCR-based method, immunohistochemistry, proteomics techniques, or any other methods known in the art, or their combination.

In another aspect, the invention provides a method for identifying a mammal that will respond therapeutically to a method of treating cancer comprising administering of an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1; (b) exposing a biological sample from the mammal to the EGFR modulator; (c) following the exposing in step (b), measuring in said biological sample the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to the said method of treating cancer.

In another aspect, the invention provides a method for identifying a mammal that will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) exposing a biological sample from the mammal to the EGFR modulator; (b) following the

exposing of step (a), measuring in said biological sample the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of the at least one biomarker measured in step (b), compared to the level of the at least one biomarker in a mammal that has not been exposed to said EGFR modulator, indicates that the mammal will respond therapeutically to said method of treating cancer.

In yet another aspect, the invention provides a method for testing or predicting whether a mammal will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises: (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1; (b) exposing the mammal to the EGFR modulator; (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

In another aspect, the invention provides a method for determining whether a compound inhibits EGFR activity in a mammal, comprising: (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of said biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the compound inhibits EGFR activity in the mammal.

In yet another aspect, the invention provides a method for determining whether a mammal has been exposed to a compound that inhibits EGFR activity, comprising (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of said biomarker measured in step (b), compared to the level of the biomarker in a mammal that has not been exposed to said compound, indicates that the mammal has been exposed to a compound that inhibits EGFR activity.

In another aspect, the invention provides a method for determining whether a mammal is responding to a compound that inhibits EGFR activity, comprising (a) exposing the mammal to the compound; and (b) following the exposing of step (a), measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, wherein a difference in the level of the at least one biomarker measured in step (b), compared to the level of the at least one biomarker in a mammal that has not been exposed to said compound, indicates that the mammal is responding to the compound that inhibits EGFR activity.

As used herein, "responding" encompasses responding by way of a biological and cellular response, as well as a clinical response (such as improved symptoms, a therapeutic effect, or an adverse event), in a mammal.

The invention also provides an isolated biomarker selected from the biomarkers of Table 1. The biomarkers of the invention comprise sequences selected from the nucleotide and amino acid sequences provided in Table 1 and the Sequence Listing, as well as fragments and variants thereof.

The invention also provides a biomarker set comprising two or more biomarkers selected from the biomarkers of Table 1.

The invention also provides kits for determining or predicting whether a patient would be susceptible or resistant to a treatment that comprises one or more EGFR modulators. The patient may have a cancer or tumor such as, for example, colorectal cancer, NSCLC, or head and neck cancer.

In one aspect, the kit comprises a suitable container that comprises one or more specialized microarrays of the invention, one or more EGFR modulators for use in testing cells from patient tissue specimens or patient samples, and instructions for use. The kit may further comprise reagents or materials for monitoring the expression of a biomarker set at the level of mRNA or protein.

In another aspect, the invention provides a kit comprising two or more biomarkers selected from the biomarkers of Table 1.

In yet another aspect, the invention provides a kit comprising at least one of an antibody and a nucleic acid for detecting the presence of at least one of the biomarkers selected from the biomarkers of Table 1. In one aspect, the kit further comprises instructions for determining whether or not a mammal will respond

therapeutically to a method of treating cancer comprising administering a compound that inhibits EGFR activity. In another aspect, the instructions comprise the steps of (a) measuring in the mammal the level of at least one biomarker selected from the biomarkers of Table 1, (b) exposing the mammal to the compound, (c) following the exposing of step (b), measuring in the mammal the level of the at least one biomarker, wherein a difference in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates that the mammal will respond therapeutically to said method of treating cancer.

The invention also provides screening assays for determining if a patient will be susceptible or resistant to treatment with one or more EGFR modulators.

The invention also provides a method of monitoring the treatment of a patient having a disease, wherein said disease is treated by a method comprising administering one or more EGFR modulators.

The invention also provides individualized genetic profiles which are necessary to treat diseases and disorders based on patient response at a molecular level.

The invention also provides specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising one or more biomarkers having expression profiles that correlate with either sensitivity or resistance to one or more EGFR modulators.

The invention also provides antibodies, including polyclonal or monoclonal, directed against one or more biomarkers of the invention.

The invention will be better understood upon a reading of the detailed description of the invention when considered in connection with the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES:

FIG. 1 illustrates a scheme used for identifying the biomarkers described herein.

FIG. 2 illustrates the expression profiling of the biomarkers described herein.

FIG. 3 (FIGS. 3A and 3B) illustrates the mRNA expression profiles of epiregulin and amphiregulin in 30 patients.

FIG. 4 illustrates the biological relationship of biomarkers described herein using Ingenuity Pathway Analysis.

FIG. 5 illustrates a comparison of a single biomarker model to multiple biomarker models.

FIG. 6 illustrates the filtering of candidate markers for cetuximab response. Expression data on 640 probe sets from 164 primary colorectal tumors was subjected to an unsupervised hierarchical clustering. The 164 tumors were divided into 3 major classes (Class 1, 2 and 3). The 640 probe sets were divided into 5 clusters (labeled A through E). Cluster A, which contains cancer antigens such as CEACAM 6 and CD24, also contains EREG and AREG. Cluster A is most highly expressed in Class 1a, which represents approximately 25% of the 164 colorectal tumor specimens.

FIG. 7 (FIGS. 7A and 7B) illustrates the mRNA levels of epiregulin and amphiregulin in 80 patients. Affymetrix mRNA levels of epiregulin (EREG, 205767_at) and amphiregulin (AREG, 205239_at) are plotted on the y axis. Subjects are ordered by best clinical response. There is a statistically significant difference in gene expression levels between the disease control group (CR, PR and SD) and the non-responder group (EREG $p = 1.474e^{-05}$, AREG $p = 2.489e^{-05}$).

FIG. 8 (FIGS. 8A and 8B) illustrates receiver operating characteristic (ROC) curves for prediction of patient response. FIG. 8A provides ROC using EREG to predict on test samples. EREG was the top single gene predictor using the discriminant function analysis, and has an area under the ROC curve (AUC) of 0.845 on the test set, indicating a high performance for prediction. FIG. 8B provides ROC using AREG to predict on the test set. The AREG gene, which was found to be coordinately regulated with the EREG gene, has an AUC of 0.815 on the test set, indicating that it too has a good prediction power as a single gene predictor.

FIG. 9 illustrates the results obtained from validation of AREG and EREG Affymetrix expression by qRT-PCR. A good correlation between the two methods (Pearson > 0.85, R2 > 0.7) was seen. High expression on Affymetrix arrays (y axis) corresponds to low ΔCt values from TaqMan qPCR assays for both AREG and EREG (x axis).

DETAILED DESCRIPTION OF THE INVENTION:

Identification of biomarkers that provide rapid and accessible readouts of efficacy, drug exposure, or clinical response is increasingly important in the clinical development of drug candidates. Embodiments of the invention include measuring changes in the levels of secreted proteins, or plasma biomarkers, which represent one category of biomarker. In one aspect, plasma samples, which represent a readily accessible source of material, serve as surrogate tissue for biomarker analysis.

The invention provides biomarkers that respond to the modulation of a specific signal transduction pathway and also correlate with EGFR modulator sensitivity or resistance. These biomarkers can be employed for predicting response to one or more EGFR modulators. In one aspect, the biomarkers of the invention are those provided in Table 1 and the Sequence Listing, including both polynucleotide and polypeptide sequences. The invention also includes nucleotide sequences that hybridize to the polynucleotides provided in Table 1.

TABLE 1 - Biomarkers

Unigene title and SEQ ID NO:	Affymetrix Description	Affymetrix Probe Set
NT5E: 5'-nucleotidase, ecto (CD73) (LOC4907) SEQ ID NOS: 1 (DNA) and 129 (amino acid)	gb:NM_002526.1 /DEF=Homo sapiens 5 nucleotidase (CD73) (NT5), mRNA. /FEA=mRNA /GEN=NT5 /PROD=5 nucleotidase /DB_XREF=gi:4505466 /UG=Hs.153952 5 nucleotidase (CD73) /FL=gb:NM_002526.1	203939_at
EREG: epiregulin (LOC2069) SEQ ID NOS: 2 (DNA) and 130 (amino acid)	gb:NM_001432.1 /DEF=Homo sapiens epiregulin (EREG), mRNA. /FEA=mRNA /GEN=EREG /PROD=epiregulin precursor /DB_XREF=gi:4557566 /UG=Hs.115263 epiregulin /FL=gb:D30783.1 gb:NM_001432.1	205767_at
AREG: amphiregulin (schwannoma-derived growth factor) (LOC374) SEQ ID NOS: 3 (DNA) and 131 (amino acid)	gb:NM_001657.1 /DEF=Homo sapiens amphiregulin (schwannoma-derived growth factor) (AREG), mRNA. /FEA=mRNA /GEN=AREG /PROD=amphiregulin (schwannoma-derived growth factor) /DB_XREF=gi:4502198 /UG=Hs.270833 amphiregulin (schwannoma-derived growth factor)	205239_at

	/FL=gb:M30704.1 gb:NM_001657.1	
LYZ: lysozyme (renal amyloidosis) (LOC4069) SEQ ID NOS: 4 (DNA) and 132 (amino acid)	Consensus includes gb:AV711904 /FEA=EST /DB_XREF=gi:10731210 /DB_XREF=est:AV711904 /CLONE=DCAAIE08 /UG=Hs.277431 Homo sapiens cDNA: FLJ23356 fis, clone HEP14919	213975_s_at
BST2: bone marrow stromal cell antigen 2 (LOC684) SEQ ID NOS: 5 (DNA) and 133 (amino acid)	gb:NM_004335.2 /DEF=Homo sapiens bone marrow stromal cell antigen 2 (BST2), mRNA. /FEA=mRNA /GEN=BST2 /PROD=bone marrow stromal cell antigen 2 /DB_XREF=gi:7262372 /UG=Hs.118110 bone marrow stromal cell antigen 2 /FL=gb:D28137.1 gb:NM_004335.2	201641_at
DUSP6: dual specificity phosphatase 6 (LOC1848) SEQ ID NOS: 6 (DNA) and 134 (amino acid)	gb:BC005047.1 /DEF=Homo sapiens, clone MGC:12852, mRNA, complete cds. /FEA=mRNA /PROD=Unknown (protein for MGC:12852) /DB_XREF=gi:13477170 /UG=Hs.180383 dual specificity phosphatase 6 /FL=gb:BC003562.1 gb:BC003143.1 gb:BC005047.1 gb:AB013382.1 gb:NM_001946.1	208893_s_at
VAV3: vav 3 oncogene (LOC10451) SEQ ID NOS: 7 (DNA) and 135 (amino acid)	gb:NM_006113.2 /DEF=Homo sapiens vav 3 oncogene (VAV3), mRNA. /FEA=mRNA /GEN=VAV3 /PROD=vav 3 oncogene /DB_XREF=gi:7262390 /UG=Hs.267659 vav 3 oncogene /FL=gb:AF067817.1 gb:AF118887.1 gb:NM_006113.2	218807_at
VAV3: vav 3 oncogene (LOC10451) SEQ ID NOS: 8 (DNA) and 136 (amino acid)	gb:AF118887.1 /DEF=Homo sapiens VAV-3 protein (VAV-3) mRNA, alternatively spliced, complete cds. /FEA=mRNA /GEN=VAV-3 /PROD=VAV-3 protein /DB_XREF=gi:4416407 /UG=Hs.267659 vav 3 oncogene /FL=gb:AF067817.1 gb:AF118887.1 gb:NM_006113.2	218806_s_at
CCL2: chemokine (C-C motif) ligand 2 (LOC6347) SEQ ID NOS: 9 (DNA) and 137 (amino acid)	Consensus includes gb:S69738.1 /DEF=MCP-1=monocyte chemotactic protein human, aortic endothelial cells, mRNA, 661 nt. /FEA=mRNA /GEN=MCP-1 /PROD=MCP-1 /DB_XREF=gi:545464	216598_s_at

	/UG=Hs.303649 small inducible cytokine A2 (monocyte chemotactic protein 1, homologous to mouse Sig-je)	
SATB2: SATB family member 2 (LOC23314) SEQ ID NOS: 10 (DNA) and 138 (amino acid)	Consensus includes gb:AB028957.1 /DEF=Homo sapiens mRNA for KIAA1034 protein, partial cds. /FEA=mRNA /GEN=KIAA1034 /PROD=KIAA1034 protein /DB_XREF=gi:5689404 /UG=Hs.12896 KIAA1034 protein	213435_at
AKAP12: A kinase (PRKA) anchor protein (gravin) 12 (LOC9590) SEQ ID NOS: 11 (DNA) and 139 (amino acid)	gb:AB003476.1 /DEF=Homo sapiens mRNA for gravin, complete cds. /FEA=mRNA /PROD=gravin /DB_XREF=gi:2081606 /UG=Hs.788 A kinase (PRKA) anchor protein (gravin) 12 /FL=gb:AB003476.1	210517_s_at
GCNT3: glucosaminyl (N-acetyl) transferase 3, mucin type (LOC9245) SEQ ID NOS: 12 (DNA) and 140 (amino acid)	gb:NM_004751.1 /DEF=Homo sapiens glucosaminyl (N-acetyl) transferase 3, mucin type (GCNT3), mRNA. /FEA=mRNA /GEN=GCNT3 /PROD=glucosaminyl (N-acetyl) transferase 3, mucintype /DB_XREF=gi:4758421 /UG=Hs.194710 glucosaminyl (N-acetyl) transferase 3, mucin type /FL=gb:AF102542.1 gb:AF038650.1 gb:NM_004751.1	219508_at
SCRN1: secernin 1 (LOC9805) SEQ ID NOS: 13 (DNA) and 141 (amino acid)	gb:NM_014766.1 /DEF=Homo sapiens KIAA0193 gene product (KIAA0193), mRNA. /FEA=mRNA /GEN=KIAA0193 /PROD=KIAA0193 gene product /DB_XREF=gi:7661983 /UG=Hs.75137 KIAA0193 gene product /FL=gb:D83777.1 gb:NM_014766.1	201462_at
FGFR3: fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism) (LOC2261) SEQ ID NOS: 14 (DNA) and 142 (amino acid)	gb:NM_000142.2 /DEF=Homo sapiens fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism) (FGFR3), transcript variant 1, mRNA. /FEA=mRNA /GEN=FGFR3 /PROD=fibroblast growth factor receptor 3, isoform 1precursor /DB_XREF=gi:13112046 /UG=Hs.1420 fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism) /FL=gb:NM_000142.2 gb:M58051.1	204379_s_at
LY96: lymphocyte antigen 96	gb:NM_015364.1 /DEF=Homo sapiens	206584_at

(LOC23643) SEQ ID NOS: 15 (DNA) and 143 (amino acid)	MD-2 protein (MD-2), mRNA. /FEA=mRNA /GEN=MD-2 /PROD=MD-2 protein /DB_XREF=gi:7662503 /UG=Hs.69328 MD-2 protein /FL=gb:AB018549.1 gb:NM_015364.1 gb:AF168121.1	
CKB: creatine kinase, brain (LOC1152) SEQ ID NOS: 16 (DNA) and 144 (amino acid)	gb:NM_001823.1 /DEF=Homo sapiens creatine kinase, brain (CKB), mRNA. /FEA=mRNA /GEN=CKB /PROD=creatine kinase, brain /DB_XREF=gi:4502850 /UG=Hs.173724 creatine kinase, brain /FL=gb:L47647.1 gb:BC001190.1 gb:BC004914.1 gb:M16364.1 gb:M16451.1 gb:NM_001823.1	200884_at
IFI16: interferon, gamma-inducible protein 16 (LOC3428) SEQ ID NOS: 17 (DNA) and 145 (amino acid)	gb:NM_005531.1 /DEF=Homo sapiens interferon, gamma-inducible protein 16 (IFI16), mRNA. /FEA=mRNA /GEN=IFI16 /PROD=interferon, gamma-inducible protein 16 /DB_XREF=gi:5031778 /UG=Hs.155530 interferon, gamma-inducible protein 16 /FL=gb:M63838.1 gb:NM_005531.1	206332_s_at
PRSS8: protease, serine, 8 (prostasin) (LOC5652) SEQ ID NOS: 18 (DNA) and 146 (amino acid)	gb:NM_002773.1 /DEF=Homo sapiens protease, serine, 8 (prostasin) (PRSS8), mRNA. /FEA=mRNA /GEN=PRSS8 /PROD=protease, serine, 8 (prostasin) /DB_XREF=gi:4506152 /UG=Hs.75799 protease, serine, 8 (prostasin) /FL=gb:BC001462.1 gb:NM_002773.1 gb:L41351.1	202525_at
IL1R2: interleukin 1 receptor, type II (LOC7850) SEQ ID NOS: 19 (DNA) and 147 (amino acid)	gb:NM_004633.1 /DEF=Homo sapiens interleukin 1 receptor, type II (IL1R2), mRNA. /FEA=mRNA /GEN=IL1R2 /PROD=interleukin 1 receptor, type II /DB_XREF=gi:4758597 /UG=Hs.25333 interleukin 1 receptor, type II /FL=gb:U74649.1 gb:NM_004633.1	205403_at
BHLHB3: basic helix-loop-helix domain containing, class B, 3 (LOC79365) SEQ ID NOS: 20 (DNA) and 148 (amino acid)	Consensus includes gb:BE857425 /FEA=EST /DB_XREF=gi:10371439 /DB_XREF=est:7f97a11.x1 /CLONE=IMAGE:3304892 /UG=Hs.33829 bHLH protein DEC2 /FL=gb:AB044088.1	221530_s_at
HLA-DRB4: major	gb:BC005312.1 /DEF=Homo sapiens,	209728_at

histocompatibility complex, class II, DR beta 4 (LOC3126) SEQ ID NOS: 21 (DNA) and 149 (amino acid)	clone MGC:12387, mRNA, complete cds. /FEA=mRNA /PROD=Unknown (protein for MGC:12387) /DB_XREF=gi:13529055 /UG=Hs.318720 Homo sapiens, clone MGC:12387, mRNA, complete cds /FL=gb:BC005312.1 gb:M16942.1	
CD163: CD163 antigen (LOC9332) SEQ ID NOS: 22 (DNA) and 150 (amino acid)	Consensus includes gb:Z22969.1 /DEF=H.sapiens mRNA for M130 antigen cytoplasmic variant 1. /FEA=mRNA /PROD=M130 antigen cytoplasmic variant 1 /DB_XREF=gi:312143 /UG=Hs.74076 CD163 antigen	215049_x_at
CD163: CD163 antigen (LOC9332) SEQ ID NOS: 23 (DNA) and 151 (amino acid)	gb:NM_004244.1 /DEF=Homo sapiens CD163 antigen (CD163), mRNA. /FEA=mRNA /GEN=CD163 /PROD=CD163 antigen /DB_XREF=gi:4758721 /UG=Hs.74076 CD163 antigen /FL=gb:NM_004244.1	203645_s_at
C13orf18: chromosome 13 open reading frame 18 (LOC80183) SEQ ID NOS: 24 (DNA) and 152 (amino acid)	gb:NM_025113.1 /DEF=Homo sapiens hypothetical protein FLJ21562 (FLJ21562), mRNA. /FEA=mRNA /GEN=FLJ21562 /PROD=hypothetical protein FLJ21562 /DB_XREF=gi:13376686 /UG=Hs.288708 hypothetical protein FLJ21562 /FL=gb:NM_025113.1	219471_at
CCL11: chemokine (C-C motif) ligand 11 (LOC6356) SEQ ID NOS: 25 (DNA) and 153 (amino acid)	gb:D49372.1 /DEF=Human mRNA for eotaxin, complete cds. /FEA=mRNA /PROD=eotaxin /DB_XREF=gi:1552240 /UG=Hs.54460 small inducible cytokine subfamily A (Cys-Cys), member 11 (eotaxin) /FL=gb:U46573.1 gb:D49372.1 gb:NM_002986.1	210133_at
SLC26A2: solute carrier family 26 (sulfate transporter), member 2 (LOC1836) SEQ ID NOS: 26 (DNA) and 154 (amino acid)	Consensus includes gb:AI025519 /FEA=EST /DB_XREF=gi:3241132 /DB_XREF=est:ov75c04.x1 /CLONE=IMAGE:1643142 /UG=Hs.29981 solute carrier family 26 (sulfate transporter), member 2 /FL=gb:NM_000112.1 gb:U14528.1	205097_at
HLA-DQB1: major histocompatibility complex, class II, DQ beta 1 (LOC3119)	gb:M32577.1 /DEF=Human MHC HLA-DQ beta mRNA, complete cds. /FEA=mRNA /GEN=HLA-DQB1 /DB_XREF=gi:188194	211656_x_at

SEQ ID NOS: 27 (DNA) and 155 (amino acid)	/FL=gb:M32577.1	
ENPP2: ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin) (LOC5168) SEQ ID NOS: 28 (DNA) and 156 (amino acid)	gb:L35594.1 /DEF=Human autotaxin mRNA, complete cds. /FEA=mRNA /PROD=autotaxin /DB_XREF=gi:537905 /UG=Hs.174185 ectonucleotide pyrophosphatasephosphodiesterase 2 (autotaxin) /FL=gb:L35594.1	209392_at
PRSS3: protease, serine, 3 (mesotrypsin) (LOC5646) SEQ ID NOS: 29 (DNA) and 157 (amino acid)	gb:NM_002770.1 /DEF=Homo sapiens protease, serine, 2 (trypsin 2) (PRSS2), mRNA. /FEA=mRNA /GEN=PRSS2 /PROD=protease, serine, 2 (trypsin 2) /DB_XREF=gi:4506146 /UG=Hs.241561 protease, serine, 2 (trypsin 2) /FL=gb:NM_002770.1 gb:M27602.1	205402_x_at
CXCR4: chemokine (C-X-C motif) receptor 4 (LOC7852) SEQ ID NOS: 30 (DNA) and 158 (amino acid)	Consensus includes gb:AJ224869 /DEF=Homo sapiens CXCR4 gene encoding receptor CXCR4 /FEA=mRNA /DB_XREF=gi:3059119 /UG=Hs.89414 chemokine (C-X-C motif), receptor 4 (fusin)	217028_at
SERPINB5: serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (LOC5268) SEQ ID NOS: 31 (DNA) and 159 (amino acid)	gb:NM_002639.1 /DEF=Homo sapiens serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), mRNA. /FEA=mRNA /GEN=SERPINB5 /PROD=serine (or cysteine) proteinase inhibitor, cladeB (ovalbumin), member 5 /DB_XREF=gi:4505788 /UG=Hs.55279 serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5 /FL=gb:NM_002639.1 gb:U04313.1	204855_at
HLA-DPB1: major histocompatibility complex, class II, DP beta 1 (LOC3115) SEQ ID NOS: 32 (DNA) and 160 (amino acid)	gb:NM_002121.1 /DEF=Homo sapiens major histocompatibility complex, class II, DP beta 1 (HLA-DPB1), mRNA. /FEA=mRNA /GEN=HLA-DPB1 /PROD=major histocompatibility complex, class II, DPbeta 1 /DB_XREF=gi:4504404 /UG=Hs.814 major histocompatibility complex, class II, DP beta 1 /FL=gb:J03041.1 gb:M57466.1 gb:M83664.1 gb:NM_002121.1 gb:M28200.1 gb:M28202.1	201137_s_at
AIF1: allograft inflammatory	Consensus includes gb:BF213829	215051_x_at

factor 1 (LOC199) SEQ ID NOS: 33 (DNA) and 161 (amino acid)	/FEA=EST /DB_XREF=gi:11107415 /DB_XREF=est:601848003F1 /CLONE=IMAGE:4078849 /UG=Hs.76364 allograft inflammatory factor 1	
IL8: interleukin 8 (LOC3576) SEQ ID NOS: 34 (DNA) and 162 (amino acid)	gb:NM_000584.1 /DEF=Homo sapiens interleukin 8 (IL8), mRNA. /FEA=mRNA /GEN=IL8 /PROD=interleukin 8 /DB_XREF=gi:10834977 /UG=Hs.624 interleukin 8 /FL=gb:NM_000584.1 gb:M17017.1 gb:M26383.1	202859_x_at
IL8: interleukin 8 (LOC3576) SEQ ID NOS: 35 (DNA) and 163 (amino acid)	gb:AF043337.1 /DEF=Homo sapiens interleukin 8 C-terminal variant (IL8) mRNA, complete cds. /FEA=mRNA /GEN=IL8 /PROD=interleukin 8 C-terminal variant /DB_XREF=gi:12641914 /UG=Hs.624 interleukin 8 /FL=gb:AF043337.1	211506_s_at
LY6G6D: lymphocyte antigen 6 complex, locus G6D (LOC58530) SEQ ID NOS: 36 (DNA) and 164 (amino acid)	gb:NM_021246.1 /DEF=Homo sapiens megakaryocyte-enhanced gene transcript 1 protein (MEGT1), mRNA. /FEA=mRNA /GEN=MEGT1 /PROD=megakaryocyte-enhanced gene transcript 1protein /DB_XREF=gi:10864054 /UG=Hs.241587 megakaryocyte-enhanced gene transcript 1 protein /FL=gb:NM_021246.1 gb:AF195764.1	207457_s_at
CYP3A5: cytochrome P450, family 3, subfamily A, polypeptide 5 (LOC1577) SEQ ID NOS: 37 (DNA) and 165 (amino acid)	gb:NM_000777.1 /DEF=Homo sapiens cytochrome P450, subfamily IIIA (niphedipine oxidase), polypeptide 5 (CYP3A5), mRNA. /FEA=mRNA /GEN=CYP3A5 /PROD=cytochrome P450, subfamily IIIA, polypeptide 5 /DB_XREF=gi:4503230 /UG=Hs.104117 cytochrome P450, subfamily IIIA (niphedipine oxidase), polypeptide 5 /FL=gb:J04813.1 gb:NM_000777.1	205765_at
CSPG2: chondroitin sulfate proteoglycan 2 (versican) (LOC1462) SEQ ID NOS: 38 (DNA) and 166 (amino acid)	Consensus includes gb:BF590263 /FEA=EST /DB_XREF=gi:11682587 /DB_XREF=est:nab22b12.x1 /CLONE=IMAGE:3266638 /UG=Hs.81800 chondroitin sulfate proteoglycan 2 (versican) /FL=gb:NM_004385.1	204619_s_at
CA9: carbonic anhydrase IX	gb:NM_001216.1 /DEF=Homo sapiens	205199_at

(LOC768) SEQ ID NOS: 39 (DNA) and 167 (amino acid)	carbonic anhydrase IX (CA9), mRNA. /FEA=mRNA /GEN=CA9 /PROD=carbonic anhydrase IX precursor /DB_XREF=gi:9955947 /UG=Hs.63287 carbonic anhydrase IX /FL=gb:NM_001216.1	
ACE2: angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (LOC59272) SEQ ID NOS: 40 (DNA) and 168 (amino acid)	gb:NM_021804.1 /DEF=Homo sapiens angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (ACE2), mRNA. /FEA=mRNA /GEN=ACE2 /PROD=angiotensin I converting enzyme(peptidyl-dipeptidase A) 2 /DB_XREF=gi:11225608 /UG=Hs.178098 angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 /FL=gb:NM_021804.1 gb:AB046569.1 gb:AF241254.1 gb:AF291820.1	219962_at
CXCL13: chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant) (LOC10563) SEQ ID NOS: 41 (DNA) and 169 (amino acid)	gb:NM_006419.1 /DEF=Homo sapiens small inducible cytokine B subfamily (Cys-X-Cys motif), member 13 (B-cell chemoattractant) (SCYB13), mRNA. /FEA=mRNA /GEN=SCYB13 /PROD=small inducible cytokine B subfamily (Cys-X-Cysmotif), member 13 (B-cell chemoattractant) /DB_XREF=gi:5453576 /UG=Hs.100431 small inducible cytokine B subfamily (Cys-X-Cys motif), member 13 (B-cell chemoattractant) /FL=gb:AF044197.1 gb:AF029894.1 gb:NM_006419.1	205242_at
COL10A1: collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia) (LOC1300) SEQ ID NOS: 42 (DNA) and 170 (amino acid)	Consensus includes gb:X98568 /DEF=H.sapiens type X collagen gene /FEA=mRNA /DB_XREF=gi:1405722 /UG=Hs.179729 collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia)	217428_s_at
CPNE1: copine I (LOC8904) SEQ ID NOS: 43 (DNA) and 171 (amino acid)	gb:NM_003915.1 /DEF=Homo sapiens copine I (CPNE1), mRNA. /FEA=mRNA /GEN=CPNE1 /PROD=copine I /DB_XREF=gi:4503012 /UG=Hs.166887 copine I /FL=gb:U83246.1 gb:NM_003915.1	206918_s_at
C13orf18: chromosome 13 open reading frame 18 (LOC80183)	Cluster Incl. AI129310:qc48a05.x1 Homo sapiens cDNA, 3 end /clone=IMAGE-1712816 /clone_end=3'	44790_s_at

SEQ ID NOS: 44 (DNA) and 172 (amino acid)	/gb=AI129310 /gi=3597824 /ug=Hs.234923 /len=811	
GREM1: gremlin 1 homolog, cysteine knot superfamily (Xenopus laevis) (LOC26585) SEQ ID NOS: 45 (DNA) and 173 (amino acid)	gb:NM_013372.1 /DEF=Homo sapiens cysteine knot superfamily 1, BMP antagonist 1 (CKTSF1B1), mRNA. /FEA=mRNA /GEN=CKTSF1B1 /PROD=cysteine knot superfamily 1, BMP antagonist 1 /DB_XREF=gi:7019348 /UG=Hs.40098 cysteine knot superfamily 1, BMP antagonist 1 /FL=gb:AF154054.1 gb:AF045800.1 gb:AF110137.2 gb:NM_013372.1	218469_at
HLA-DQB1: major histocompatibility complex, class II, DQ beta 1 (LOC3119) SEQ ID NOS: 46 (DNA) and 174 (amino acid)	gb:M17955.1 /DEF=Human MHC class II HLA-DQ-beta mRNA, complete cds. /FEA=mRNA /DB_XREF=gi:188178 /UG=Hs.73931 major histocompatibility complex, class II, DQ beta 1 /FL=gb:M33907.1 gb:M17955.1 gb:M17563.1 gb:M26042.1 gb:M20432.1 gb:M16996.1	209823_x_at
TCN1: transcobalamin I (vitamin B12 binding protein, R binder family) (LOC6947) SEQ ID NOS: 47 (DNA) and 175 (amino acid)	gb:NM_001062.1 /DEF=Homo sapiens transcobalamin I (vitamin B12 binding protein, R binder family) (TCN1), mRNA. /FEA=mRNA /GEN=TCN1 /PROD=transcobalamin I (vitamin B12 binding protein, Rbinder family) /DB_XREF=gi:4507406 /UG=Hs.2012 transcobalamin I (vitamin B12 binding protein, R binder family) /FL=gb:J05068.1 gb:NM_001062.1	205513_at
PIGR: polymeric immunoglobulin receptor (LOC5284) SEQ ID NOS: 48 (DNA) and 176 (amino acid)	gb:NM_002644.1 /DEF=Homo sapiens polymeric immunoglobulin receptor (PIGR), mRNA. /FEA=mRNA /GEN=PIGR /PROD=polymeric immunoglobulin receptor /DB_XREF=gi:11342673 /UG=Hs.288579 polymeric immunoglobulin receptor /FL=gb:NM_002644.1	204213_at
COL10A1: collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia) (LOC1300) SEQ ID NOS: 49 (DNA) and 177 (amino acid)	Consensus includes gb:AI376003 /FEA=EST /DB_XREF=gi:4175993 /DB_XREF=est:tc30d11.x1 /CLONE=IMAGE:2066133 /UG=Hs.179729 collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia)	205941_s_at

	/FL=gb:NM_000493.1	
KCTD12: potassium channel tetramerisation domain containing 12 (LOC115207) SEQ ID NOS: 50 (DNA) and 178 (amino acid)	Consensus includes gb:AI718937 /FEA=EST /DB_XREF=gi:5036193 /DB_XREF=est:as50b04.x1 /CLONE=IMAGE:2320591 /UG=Hs.109438 Homo sapiens clone 24775 mRNA sequence	212192_at
LCK: lymphocyte-specific protein tyrosine kinase (LOC3932) SEQ ID NOS: 51 (DNA) and 179 (amino acid)	gb:NM_005356.1 /DEF=Homo sapiens lymphocyte-specific protein tyrosine kinase (LCK), mRNA. /FEA=mRNA /GEN=LCK /PROD=lymphocyte-specific protein tyrosine kinase /DB_XREF=gi:4885448 /UG=Hs.1765 lymphocyte-specific protein tyrosine kinase /FL=gb:M36881.1 gb:U07236.1 gb:NM_005356.1	204891_s_at
LAPTM4B: lysosomal associated protein transmembrane 4 beta (LOC55353) SEQ ID NOS: 52 (DNA) and 180 (amino acid)	gb:NM_018407.1 /DEF=Homo sapiens putative integral membrane transporter (LC27), mRNA. /FEA=mRNA /GEN=LC27 /PROD=putative integral membrane transporter /DB_XREF=gi:8923827 /FL=gb:NM_018407.1	208029_s_at
CEACAM5: carcinoembryonic antigen-related cell adhesion molecule 5 (LOC1048) SEQ ID NOS: 53 (DNA) and 181 (amino acid)	gb:NM_004363.1 /DEF=Homo sapiens carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), mRNA. /FEA=mRNA /GEN=CEACAM5 /PROD=carcinoembryonic antigen-related cell adhesionmolecule 5 /DB_XREF=gi:11386170 /UG=Hs.220529 carcinoembryonic antigen-related cell adhesion molecule 5 /FL=gb:NM_004363.1 gb:M29540.1	201884_at
LDHB: lactate dehydrogenase B (LOC3945) SEQ ID NOS: 54 (DNA) and 182 (amino acid)	gb:NM_002300.1 /DEF=Homo sapiens lactate dehydrogenase B (LDHB), mRNA. /FEA=mRNA /GEN=LDHB /PROD=lactate dehydrogenase B /DB_XREF=gi:4557031 /UG=Hs.234489 lactate dehydrogenase B /FL=gb:BC002362.1 gb:NM_002300.1	201030_x_at
IFI27: interferon, alpha-inducible protein 27 (LOC3429) SEQ ID NOS: 55 (DNA) and 183 (amino acid)	gb:NM_005532.1 /DEF=Homo sapiens interferon, alpha-inducible protein 27 (IFI27), mRNA. /FEA=mRNA /GEN=IFI27 /PROD=interferon, alpha-inducible protein 27 /DB_XREF=gi:5031780	202411_at

	/UG=Hs.278613 interferon, alpha-inducible protein 27 /FL=gb:NM_005532.1	
EPHB2: EphB2 (LOC2048) SEQ ID NOS: 56 (DNA) and 184 (amino acid)	gb:D31661.1 /DEF=Human mRNA for tyrosine kinase, complete cds. /FEA=mRNA /GEN=ERK /PROD=tyrosine kinase precursor /DB_XREF=gi:495677 /UG=Hs.125124 EphB2 /FL=gb:D31661.1	211165_x_at
ACACA: acetyl-Coenzyme A carboxylase alpha (LOC31) SEQ ID NOS: 57 (DNA) and 185 (amino acid)	Consensus includes gb:BE855983 /FEA=EST /DB_XREF=gi:10368561 /DB_XREF=est:7f85g11.x1 /CLONE=IMAGE:3303812 /UG=Hs.7232 acetyl-Coenzyme A carboxylase alpha /FL=gb:NM_000664.1 gb:U19822.1	212186_at
CD14: CD14 antigen (LOC929) SEQ ID NOS: 58 (DNA) and 186 (amino acid)	gb:NM_000591.1 /DEF=Homo sapiens CD14 antigen (CD14), mRNA. /FEA=mRNA /GEN=CD14 /PROD=CD14 antigen precursor /DB_XREF=gi:4557416 /UG=Hs.75627 CD14 antigen /FL=gb:M86511.1 gb:AF097942.1 gb:NM_000591.1	201743_at
ABHD2: abhydrolase domain containing 2 (LOC11057) SEQ ID NOS: 59 (DNA) and 187 (amino acid)	Cluster Incl. AI832249:td14g10.x1 Homo sapiens cDNA, 3 end /clone=IMAGE-2075682 /clone_end=3' /gb=AI832249 /gi=5452920 /ug=Hs.211522 /len=545	87100_at
TNFRSF6B: tumor necrosis factor receptor superfamily, member 6b, decoy (LOC8771) SEQ ID NOS: 60 (DNA) and 188 (amino acid)	gb:NM_003823.1 /DEF=Homo sapiens tumor necrosis factor receptor superfamily, member 6b, decoy (TNFRSF6B), mRNA. /FEA=mRNA /GEN=TNFRSF6B /PROD=decoy receptor 3 /DB_XREF=gi:4507584 /UG=Hs.278556 tumor necrosis factor receptor superfamily, member 6b, decoy /FL=gb:AF104419.1 gb:NM_003823.1 gb:AF134240.1 gb:AF217794.1	206467_x_at
GREM1: gremlin 1 homolog, cysteine knot superfamily (Xenopus laevis) (LOC26585) SEQ ID NOS: 61 (DNA) and 189 (amino acid)	gb:AF154054.1 /DEF=Homo sapiens DRM (DRM) mRNA, complete cds. /FEA=mRNA /GEN=DRM /PROD=DRM /DB_XREF=gi:10863087 /UG=Hs.40098 cysteine knot superfamily 1, BMP antagonist 1	218468_s_at

	/FL=gb:AF154054.1 gb:AF045800.1 gb:AF110137.2 gb:NM_013372.1	
ACE2: angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (LOC59272) SEQ ID NOS: 62 (DNA) and 190 (amino acid)	Consensus includes gb:AK026461.1 /DEF=Homo sapiens cDNA: FLJ22808 fis, clone KAIA2925. /FEA=mRNA /DB_XREF=gi:10439331 /UG=Hs.178098 angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	222257_s_at
COL5A2: collagen, type V, alpha 2 (LOC1290) SEQ ID NOS: 63 (DNA) and 191 (amino acid)	Consensus includes gb:NM_000393.1 /DEF=Homo sapiens collagen, type V, alpha 2 (COL5A2), mRNA. /FEA=CDS /GEN=COL5A2 /PROD=collagen, type V, alpha 2 /DB_XREF=gi:4502958 /UG=Hs.82985 collagen, type V, alpha 2 /FL=gb:NM_000393.1	221730_at
CXCL9: chemokine (C-X-C motif) ligand 9 (LOC4283) SEQ ID NOS: 64 (DNA) and 192 (amino acid)	gb:NM_002416.1 /DEF=Homo sapiens monokine induced by gamma interferon (MIG), mRNA. /FEA=mRNA /GEN=MIG /PROD=monokine induced by gamma interferon /DB_XREF=gi:4505186 /UG=Hs.77367 monokine induced by gamma interferon /FL=gb:NM_002416.1	203915_at
HOXC6: homeo box C6 (LOC3223) SEQ ID NOS: 65 (DNA) and 193 (amino acid)	gb:NM_004503.1 /DEF=Homo sapiens homeo box C6 (HOXC6), mRNA. /FEA=mRNA /GEN=HOXC6 /PROD=homeo box C6 /DB_XREF=gi:4758553 /UG=Hs.820 homeo box C6 /FL=gb:NM_004503.1	206858_s_at
KCNMA1: potassium large conductance calcium-activated channel, subfamily M, alpha member 1 (LOC3778) SEQ ID NOS: 66 (DNA) and 194 (amino acid)	gb:U11058.2 /DEF=Homo sapiens large conductance calcium- and voltage-dependent potassium channel alpha subunit (MaxiK) mRNA, complete cds. /FEA=mRNA /GEN=MaxiK /PROD=large conductance calcium- and voltage-dependentpotassium channel alpha subunit /DB_XREF=gi:7914977 /UG=Hs.89463 potassium large conductance calcium-activated channel, subfamily M, alpha member 1 /FL=gb:AF025999.1 gb:NM_002247.1 gb:AF118141.1 gb:U13913.1 gb:U11717.1 gb:U23767.1 gb:U11058.2	221584_s_at

MMP1: matrix metalloproteinase 1 (interstitial collagenase) (LOC4312) SEQ ID NOS: 67 (DNA) and 195 (amino acid)	gb:NM_002421.2 /DEF=Homo sapiens matrix metalloproteinase 1 (interstitial collagenase) (MMP1), mRNA. /FEA=mRNA /GEN=MMP1 /PROD=matrix metalloproteinase 1 preproprotein /DB_XREF=gi:13027798 /UG=Hs.83169 matrix metalloproteinase 1 (interstitial collagenase) /FL=gb:NM_002421.2 gb:M13509.1	204475_at
PLCB4: phospholipase C, beta 4 (LOC5332) SEQ ID NOS: 68 (DNA) and 196 (amino acid)	Consensus includes gb:AL535113 /FEA=EST /DB_XREF=gi:12798606 /DB_XREF=est:AL535113 /CLONE=CS0DF008YC23 (3 prime) /UG=Hs.283006 phospholipase C, beta 4 /FL=gb:NM_000933.1 gb:L41349.1	203895_at
PTPRD: protein tyrosine phosphatase, receptor type, D (LOC5789) SEQ ID NOS: 69 (DNA) and 197 (amino acid)	Consensus includes gb:BF062299 /FEA=EST /DB_XREF=gi:10821197 /DB_XREF=est:7k76c03.x1 /CLONE=IMAGE:3481325 /UG=Hs.323079 Homo sapiens mRNA; cDNA DKFZp564P116 (from clone DKFZp564P116)	214043_at
KCNK1: potassium channel, subfamily K, member 1 (LOC3775) SEQ ID NOS: 70 (DNA) and 198 (amino acid)	gb:U90065.1 /DEF=Human potassium channel KCNO1 mRNA, complete cds. /FEA=mRNA /PROD=potassium channel KCNO1 /DB_XREF=gi:1916294 /UG=Hs.79351 potassium channel, subfamily K, member 1 (TWIK-1) /FL=gb:U33632.1 gb:U90065.1 gb:U76996.1 gb:NM_002245.1	204678_s_at
ALOX5: arachidonate 5-lipoxygenase (LOC240) SEQ ID NOS: 71 (DNA) and 199 (amino acid)	gb:NM_000698.1 /DEF=Homo sapiens arachidonate 5-lipoxygenase (ALOX5), mRNA. /FEA=mRNA /GEN=ALOX5 /PROD=arachidonate 5-lipoxygenase /DB_XREF=gi:4502056 /UG=Hs.89499 arachidonate 5-lipoxygenase /FL=gb:J03600.1 gb:J03571.1 gb:NM_000698.1	204446_s_at
CXCL10: chemokine (C-X-C motif) ligand 10 (LOC3627) SEQ ID NOS: 72 (DNA) and 200 (amino acid)	gb:NM_001565.1 /DEF=Homo sapiens small inducible cytokine subfamily B (Cys-X-Cys), member 10 (SCYB10), mRNA. /FEA=mRNA /GEN=SCYB10 /PROD=interferon gamma-induced precursor /DB_XREF=gi:4504700 /UG=Hs.2248	204533_at

	small inducible cytokine subfamily B (Cys-X-Cys), member 10 /FL=gb:NM_001565.1	
TMPRSS2: transmembrane protease, serine 2 (LOC7113) SEQ ID NOS: 73 (DNA) and 201 (amino acid)	gb:AF270487.1 /DEF=Homo sapiens androgen-regulated serine protease TMPRSS2 precursor (TMPRSS2) mRNA, complete cds. /FEA=mRNA /GEN=TMPRSS2 /PROD=androgen-regulated serine protease TMPRSS2precursor /DB_XREF=gi:13540003 /FL=gb:AF270487.1	211689_s_at
PRG1: proteoglycan 1, secretory granule (LOC5552) SEQ ID NOS: 74 (DNA) and 202 (amino acid)	gb:J03223.1 /DEF=Human secretory granule proteoglycan peptide core mRNA, complete cds. /FEA=mRNA /GEN=PRG1 /DB_XREF=gi:190419 /UG=Hs.1908 proteoglycan 1, secretory granule /FL=gb:J03223.1 gb:NM_002727.1	201858_s_at
HLA-DQA1: major histocompatibility complex, class II, DQ alpha 1 (LOC3117) SEQ ID NOS: 75 (DNA) and 203 (amino acid)	Consensus includes gb:BG397856 /FEA=EST /DB_XREF=gi:13291304 /DB_XREF=est:602438950F1 /CLONE=IMAGE:4564956 /UG=Hs.198253 major histocompatibility complex, class II, DQ alpha 1	212671_s_at
NR4A2: nuclear receptor subfamily 4, group A, member 2 (LOC4929) SEQ ID NOS: 76 (DNA) and 204 (amino acid)	Consensus includes gb:S77154.1 /DEF=TINUR= NGFI-Bnur77 beta-type transcription factor homolog human, T lymphoid cell line, PEER, mRNA, 2469 nt. /FEA=mRNA /GEN=TINUR /DB_XREF=gi:913966 /UG=Hs.82120 nuclear receptor subfamily 4, group A, member 2	216248_s_at
KCTD12: potassium channel tetramerisation domain containing 12 (LOC115207) SEQ ID NOS: 77 (DNA) and 205 (amino acid)	Consensus includes gb:AA551075 /FEA=EST /DB_XREF=gi:2321327 /DB_XREF=est:nk74h06.s1 /CLONE=IMAGE:1019291 /UG=Hs.109438 Homo sapiens clone 24775 mRNA sequence	212188_at
RARRES3: retinoic acid receptor responder (tazarotene induced) 3 (LOC5920) SEQ ID NOS: 78 (DNA) and 206 (amino acid)	gb:NM_004585.2 /DEF=Homo sapiens retinoic acid receptor responder (tazarotene induced) 3 (RARRES3), mRNA. /FEA=mRNA /GEN=RARRES3 /PROD=retinoic acid receptor responder (tazaroteneinduced) 3 /DB_XREF=gi:8051633	204070_at

	/UG=Hs.17466 retinoic acid receptor responder (tazarotene induced) 3 /FL=gb:AF060228.1 gb:AF092922.1 gb:NM_004585.2 gb:AB030815.1	
LDHB: lactate dehydrogenase B (LOC3945) SEQ ID NOS: 79 (DNA) and 207 (amino acid)	Consensus includes gb:BE042354 /FEA=EST /DB_XREF=gi:8359407 /DB_XREF=est:ho19b09.x1 /CLONE=IMAGE:3037817 /UG=Hs.234489 lactate dehydrogenase B	213564_x_at
CLECSF2: C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced) (LOC9976) SEQ ID NOS: 80 (DNA) and 208 (amino acid)	gb:BC005254.1 /DEF=Homo sapiens, Similar to C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced), clone MGC:12289, mRNA, complete cds. /FEA=mRNA /PROD=Similar to C-type (calcium dependent,carbohydrate-recognition domain) lectin, superfamilymember 2 (activation-induced) /DB_XREF=gi:13528920 /UG=Hs.85201 C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced) /FL=gb:BC005254.1 gb:AB015628.1 gb:NM_005127.1	209732_at
FLNA: filamin A, alpha (actin binding protein 280) (LOC2316) SEQ ID NOS: 81 (DNA) and 209 (amino acid)	Consensus includes gb:AW051856 /FEA=EST /DB_XREF=gi:5914215 /DB_XREF=est:wz04a05.x1 /CLONE=IMAGE:2557040 /UG=Hs.195464 filamin A, alpha (actin-binding protein-280)	213746_s_at
CXCL5: chemokine (C-X-C motif) ligand 5 (LOC6374) SEQ ID NOS: 82 (DNA) and 210 (amino acid)	Consensus includes gb:AK026546.1 /DEF=Homo sapiens cDNA: FLJ22893 fis, clone KAT04792. /FEA=mRNA /DB_XREF=gi:10439427 /UG=Hs.287716 Homo sapiens cDNA: FLJ22893 fis, clone KAT04792	214974_x_at
AEBP1: AE binding protein 1 (LOC165) SEQ ID NOS: 83 (DNA) and 211 (amino acid)	gb:NM_001129.2 /DEF=Homo sapiens AE-binding protein 1 (AEBP1), mRNA. /FEA=mRNA /GEN=AEBP1 /PROD=adipocyte enhancer binding protein 1 precursor /DB_XREF=gi:4755145 /UG=Hs.118397 AE-binding protein 1 /FL=gb:D86479.1 gb:AF053944.1 gb:NM_001129.2	201792_at

BGN: biglycan (LOC633) SEQ ID NOS: 84 (DNA) and 212 (amino acid)	Consensus includes gb:AA845258 /FEA=EST /DB_XREF=gi:2931709 /DB_XREF=est:ak84a11.s1 /CLONE=IMAGE:1414556 /UG=Hs.821 biglycan	213905_x_at
SULF1: sulfatase 1 (LOC23213) SEQ ID NOS: 85 (DNA) and 213 (amino acid)	Consensus includes gb:AI479175 /FEA=EST /DB_XREF=gi:4372343 /DB_XREF=est:tm55c05.x1 /CLONE=IMAGE:2162024 /UG=Hs.70823 KIAA1077 protein	212353_at
COL6A2: collagen, type VI, alpha 2 (LOC1292) SEQ ID NOS: 86 (DNA) and 214 (amino acid)	gb:AY029208.1 /DEF=Homo sapiens type VI collagen alpha 2 chain precursor (COL6A2) mRNA, complete cds, alternatively spliced. /FEA=mRNA /GEN=COL6A2 /PROD=type VI collagen alpha 2 chain precursor /DB_XREF=gi:13603393 /UG=Hs.159263 collagen, type VI, alpha 2 /FL=gb:AY029208.1	209156_s_at
THBS2: thrombospondin 2 (LOC7058) SEQ ID NOS: 87 (DNA) and 215 (amino acid)	gb:NM_003247.1 /DEF=Homo sapiens thrombospondin 2 (THBS2), mRNA. /FEA=mRNA /GEN=THBS2 /PROD=thrombospondin 2 /DB_XREF=gi:4507486 /UG=Hs.108623 thrombospondin 2 /FL=gb:L12350.1 gb:NM_003247.1	203083_at
PLCB4: phospholipase C, beta 4 (LOC5332) SEQ ID NOS: 88 (DNA) and 216 (amino acid)	gb:NM_000933.1 /DEF=Homo sapiens phospholipase C, beta 4 (PLCB4), mRNA. /FEA=mRNA /GEN=PLCB4 /PROD=phospholipase C, beta 4 /DB_XREF=gi:4505866 /UG=Hs.283006 phospholipase C, beta 4 /FL=gb:NM_000933.1 gb:L41349.1	203896_s_at
CALD1: caldesmon 1 (LOC800) SEQ ID NOS: 89 (DNA) and 217 (amino acid)	gb:NM_004342.2 /DEF=Homo sapiens caldesmon 1 (CALD1), mRNA. /FEA=mRNA /GEN=CALD1 /PROD=caldesmon 1 /DB_XREF=gi:11091984 /UG=Hs.325474 caldesmon 1 /FL=gb:NM_004342.2 gb:M64110.1	201617_x_at
NGFRAP1: nerve growth factor receptor (TNFRSF16) associated protein 1 (LOC27018) SEQ ID NOS: 90 (DNA) and 218 (amino acid)	gb:NM_014380.1 /DEF=Homo sapiens p75NTR-associated cell death executor; ovarian granulosa cell protein (13kD) (DXS6984E), mRNA. /FEA=mRNA /GEN=DXS6984E /PROD=p75NTR-associated cell death executor; ovariangranulosa cell protein (13kD) /DB_XREF=gi:7657043	217963_s_at

	/UG=Hs.17775 p75NTR-associated cell death executor; ovarian granulosa cell protein (13kD) /FL=gb:NM_014380.1 gb:AF187064.1	
IFI16: interferon, gamma-inducible protein 16 (LOC3428) SEQ ID NOS: 91 (DNA) and 219 (amino acid)	Consensus includes gb:BG256677 /FEA=EST /DB_XREF=gi:12766493 /DB_XREF=est:602370865F1 /CLONE=IMAGE:4478872 /UG=Hs.155530 interferon, gamma-inducible protein 16 /FL=gb:AF208043.1	208965_s_at
RAB31: RAB31, member RAS oncogene family (LOC11031) SEQ ID NOS: 92 (DNA) and 220 (amino acid)	gb:NM_006868.1 /DEF=Homo sapiens RAB31, member RAS oncogene family (RAB31), mRNA. /FEA=mRNA /GEN=RAB31 /PROD=RAB31, member RAS oncogene family /DB_XREF=gi:5803130 /UG=Hs.223025 RAB31, member RAS oncogene family /FL=gb:AF234995.1 gb:BC001148.1 gb:U59877.1 gb:U57091.1 gb:NM_006868.1 gb:AF183421.1	217763_s_at
COL5A1: collagen, type V, alpha 1 (LOC1289) SEQ ID NOS: 93 (DNA) and 221 (amino acid)	Consensus includes gb:AI130969 /FEA=EST /DB_XREF=gi:3600985 /DB_XREF=est:qc15e01.x1 /CLONE=IMAGE:1709688 /UG=Hs.146428 collagen, type V, alpha 1 /FL=gb:M76729.1 gb:D90279.1 gb:NM_000093.1	203325_s_at
KLK10: kallikrein 10 (LOC5655) SEQ ID NOS: 94 (DNA) and 222 (amino acid)	gb:BC002710.1 /DEF=Homo sapiens, kallikrein 10, clone MGC:3667, mRNA, complete cds. /FEA=mRNA /PROD=kallikrein 10 /DB_XREF=gi:12803744 /UG=Hs.69423 kallikrein 10 /FL=gb:BC002710.1	209792_s_at
PCP4: Purkinje cell protein 4 (LOC5121) SEQ ID NOS: 95 (DNA) and 223 (amino acid)	gb:NM_006198.1 /DEF=Homo sapiens Purkinje cell protein 4 (PCP4), mRNA. /FEA=mRNA /GEN=PCP4 /PROD=Purkinje cell protein 4 /DB_XREF=gi:5453857 /UG=Hs.80296 Purkinje cell protein 4 /FL=gb:U52969.1 gb:NM_006198.1	205549_at
NR4A2: nuclear receptor subfamily 4, group A, member 2 (LOC4929) SEQ ID NOS: 96 (DNA) and	gb:NM_006186.1 /DEF=Homo sapiens nuclear receptor subfamily 4, group A, member 2 (NR4A2), mRNA. /FEA=mRNA /GEN=NR4A2 /PROD=nuclear receptor subfamily 4,	204622_x_at

224 (amino acid)	group A, member 2 /DB_XREF=gi:5453821 /UG=Hs.82120 nuclear receptor subfamily 4, group A, member 2 /FL=gb:NM_006186.1	
IGFBP3: insulin-like growth factor binding protein 3 (LOC3486) SEQ ID NOS: 97 (DNA) and 225 (amino acid)	gb:M31159.1 /DEF=Human growth hormone-dependent insulin-like growth factor-binding protein mRNA, complete cds. /FEA=mRNA /GEN=IGFBP1 /DB_XREF=gi:183115 /UG=Hs.77326 insulin-like growth factor binding protein 3 /FL=gb:BC000013.1 gb:M31159.1	210095_s_at
STAT1: signal transducer and activator of transcription 1, 91kDa (LOC6772) SEQ ID NOS: 98 (DNA) and 226 (amino acid)	gb:BC002704.1 /DEF=Homo sapiens, Similar to signal transducer and activator of transcription 1, 91kD, clone MGC:3493, mRNA, complete cds. /FEA=mRNA /PROD=Similar to signal transducer and activator oftranscription 1, 91kD /DB_XREF=gi:12803734 /UG=Hs.21486 signal transducer and activator of transcription 1, 91kD /FL=gb:BC002704.1	209969_s_at
CYP1B1: cytochrome P450, family 1, subfamily B, polypeptide 1 (LOC1545) SEQ ID NOS: 99 (DNA) and 227 (amino acid)	Consensus includes gb:AU144855 /FEA=EST /DB_XREF=gi:11006376 /DB_XREF=est:AU144855 /CLONE=HEMBA1003161 /UG=Hs.154654 cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1 (glaucoma 3, primary infantile) /FL=gb:NM_000104.2 gb:U03688.1	202436_s_at
COL1A1: collagen, type I, alpha 1 (LOC1277) SEQ ID NOS: 100 (DNA) and 228 (amino acid)	Consensus includes gb:AI743621 /FEA=EST /DB_XREF=gi:5111909 /DB_XREF=est:wg51h09.x1 /CLONE=IMAGE:2368673 /UG=Hs.172928 collagen, type I, alpha 1 /FL=gb:NM_000088.1	202311_s_at
DKFZP434F0318: hypothetical protein DKFZp434F0318 (LOC81575) SEQ ID NOS: 101 (DNA) and 229 (amino acid)	gb:NM_030817.1 /DEF=Homo sapiens hypothetical protein DKFZp434F0318 (DKFZP434F0318), mRNA. /FEA=mRNA /GEN=DKFZP434F0318 /PROD=hypothetical protein DKFZp434F0318 /DB_XREF=gi:13540611 /FL=gb:NM_030817.1	221031_s_at
TUBA3: tubulin, alpha 3	gb:AF141347.1 /DEF=Homo sapiens	209118_s_at

(LOC7846) SEQ ID NOS: 102 (DNA) and 230 (amino acid)	hum-a-tub2 alpha-tubulin mRNA, complete cds. /FEA=mRNA /PROD=alpha-tubulin /DB_XREF=gi:4929133 /UG=Hs.272897 Tubulin, alpha, brain-specific /FL=gb:AF141347.1 gb:NM_006009.1	
GZMB: granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) (LOC3002) SEQ ID NOS: 103 (DNA) and 231 (amino acid)	gb:J03189.1 /DEF=Human proteolytic serine esterase-like protein (SECT) gene, complete cds. /FEA=mRNA /DB_XREF=gi:338010 /UG=Hs.1051 granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) /FL=gb:J04071.1 gb:J03189.1 gb:M17016.1 gb:NM_004131.2	210164_at
ROBO1: roundabout, axon guidance receptor, homolog 1 (Drosophila) (LOC6091) SEQ ID NOS: 104 (DNA) and 232 (amino acid)	Consensus includes gb:BF059159 /FEA=EST /DB_XREF=gi:10813055 /DB_XREF=est:7k66g04.x1 /CLONE=IMAGE:3480391 /UG=Hs.301198 roundabout (axon guidance receptor, Drosophila) homolog 1 /FL=gb:AF040990.1 gb:NM_002941.1	213194_at
CHGA: chromogranin A (parathyroid secretory protein 1) (LOC1113) SEQ ID NOS: 105 (DNA) and 233 (amino acid)	gb:NM_001275.2 /DEF=Homo sapiens chromogranin A (parathyroid secretory protein 1) (CHGA), mRNA. /FEA=mRNA /GEN=CHGA /PROD=chromogranin A /DB_XREF=gi:10800418 /UG=Hs.172216 chromogranin A (parathyroid secretory protein 1) /FL=gb:NM_001275.2 gb:BC001059.1 gb:J03483.1 gb:J03915.1	204697_s_at
SLC7A8: solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 (LOC23428) SEQ ID NOS: 106 (DNA) and 234 (amino acid)	gb:NM_012244.1 /DEF=Homo sapiens solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 (SLC7A8), mRNA. /FEA=mRNA /GEN=SLC7A8 /PROD=solute carrier family 7 (cationic amino acidtransporter, y+ system), member 8 /DB_XREF=gi:6912669 /UG=Hs.22891 solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 /FL=gb:AB037669.1 gb:AF171669.1 gb:NM_012244.1	202752_x_at
GPA33: glycoprotein A33 (transmembrane) (LOC10223)	gb:NM_005814.1 /DEF=Homo sapiens glycoprotein A33 (transmembrane)	205929_at

SEQ ID NOS: 107 (DNA) and 235 (amino acid)	(GPA33), mRNA. /FEA=mRNA /GEN=GPA33 /PROD=transmembrane glycoprotein A33 precursor /DB_XREF=gi:5031560 /UG=Hs.143131 glycoprotein A33 (transmembrane) /FL=gb:U79725.1 gb:NM_005814.1	
QPRT: quinolinate phosphoribosyltransferase (nicotinate-nucleotide pyrophosphorylase (carboxylating)) (LOC23475) SEQ ID NOS: 108 (DNA) and 236 (amino acid)	gb:NM_014298.2 /DEF=Homo sapiens quinolinate phosphoribosyltransferase (nicotinate-nucleotide pyrophosphorylase (carboxylating)) (QPRT), mRNA. /FEA=mRNA /GEN=QPRT /PROD=quinolinate phosphoribosyltransferase /DB_XREF=gi:9257236 /UG=Hs.8935 quinolinate phosphoribosyltransferase (nicotinate-nucleotide pyrophosphorylase (carboxylating)) /FL=gb:D78177.1 gb:BC005060.1 gb:NM_014298.2	204044_at
DDC: dopa decarboxylase (aromatic L-amino acid decarboxylase) (LOC1644) SEQ ID NOS: 109 (DNA) and 237 (amino acid)	gb:NM_000790.1 /DEF=Homo sapiens dopa decarboxylase (aromatic L-amino acid decarboxylase) (DDC), mRNA. /FEA=mRNA /GEN=DDC /PROD=dopa decarboxylase (aromatic L-amino aciddecarboxylase) /DB_XREF=gi:4503280 /UG=Hs.150403 dopa decarboxylase (aromatic L-amino acid decarboxylase) /FL=gb:BC000485.1 gb:M76180.1 gb:M88700.1 gb:NM_000790.1	205311_at
COL11A1: collagen, type XI, alpha 1 (LOC1301) SEQ ID NOS: 110 (DNA) and 238 (amino acid)	gb:NM_001854.1 /DEF=Homo sapiens collagen, type XI, alpha 1 (COL11A1), mRNA. /FEA=mRNA /GEN=COL11A1 /PROD=collagen, type XI, alpha 1 /DB_XREF=gi:4502938 /UG=Hs.82772 collagen, type XI, alpha 1 /FL=gb:J04177.1 gb:NM_001854.1	204320_at
C2orf23: chromosome 2 open reading frame 23 (LOC65055) SEQ ID NOS: 111 (DNA) and 239 (amino acid)	Consensus includes gb:BE535746 /FEA=EST /DB_XREF=gi:9764391 /DB_XREF=est:601060419F1 /CLONE=IMAGE:3446788 /UG=Hs.7358 hypothetical protein FLJ13110 /FL=gb:NM_022912.1	204364_s_at
SULF1: sulfatase 1 (LOC23213) SEQ ID NOS: 112 (DNA) and	Consensus includes gb:BE500977 /FEA=EST /DB_XREF=gi:9703385 /DB_XREF=est:7a33h02.x1	212354_at

240 (amino acid)	/CLONE=IMAGE:3220563 /UG=Hs.70823 KIAA1077 protein	
PCOLCE: procollagen C-endopeptidase enhancer (LOC5118) SEQ ID NOS: 113 (DNA) and 241 (amino acid)	gb:NM_002593.2 /DEF=Homo sapiens procollagen C-endopeptidase enhancer (PCOLCE), mRNA. /FEA=mRNA /GEN=PCOLCE /PROD=procollagen C-endopeptidase enhancer /DB_XREF=gi:7262388 /UG=Hs.202097 procollagen C-endopeptidase enhancer /FL=gb:BC000574.1 gb:AB008549.1 gb:L33799.1 gb:NM_002593.2	202465_at
C14orf78: chromosome 14 open reading frame 78 (LOC113146) SEQ ID NOS: 114 (DNA) and 242 (amino acid)	Consensus includes gb:AI935123 /FEA=EST /DB_XREF=gi:5673993 /DB_XREF=est:wp13h09.x1 /CLONE=IMAGE:2464769 /UG=Hs.57548 ESTs	212992_at
CXCR4: chemokine (C-X-C motif) receptor 4 (LOC7852) SEQ ID NOS: 115 (DNA) and 243 (amino acid)	gb:L01639.1 /DEF=Human (clone HSY3RR) neuropeptide Y receptor (NPYR) mRNA, complete cds. /FEA=mRNA /GEN=NPYR /PROD=neuropeptide Y receptor /DB_XREF=gi:189313 /UG=Hs.89414 chemokine (C-X-C motif), receptor 4 (fusin) /FL=gb:L01639.1 gb:AF025375.1 gb:M99293.1 gb:L06797.1 gb:NM_003467.1 gb:AF147204.1	209201_x_at
CSPG2: chondroitin sulfate proteoglycan 2 (versican) (LOC1462) SEQ ID NOS: 116 (DNA) and 244 (amino acid)	Consensus includes gb:R94644 /FEA=EST /DB_XREF=gi:970039 /DB_XREF=est:yq42a12.r1 /CLONE=IMAGE:198430 /UG=Hs.306542 Homo sapiens versican Vint isoform, mRNA, partial cds	215646_s_at
SERPINF1: serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 (LOC5176) SEQ ID NOS: 117 (DNA) and 245 (amino acid)	gb:NM_002615.1 /DEF=Homo sapiens serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 (SERPINF1), mRNA. /FEA=mRNA /GEN=SERPINF1 /PROD=serine (or cysteine) proteinase inhibitor, cladeF (alpha-2 antiplasmin, pigment epithelium derivedfactor), member 1 /DB_XREF=gi:4505708 /UG=Hs.173594 serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium	202283_at

	derived factor), member 1 /FL=gb:M90439.1 gb:BC000522.1 gb:M76979.1 gb:NM_002615.1	
SPON1: spondin 1, extracellular matrix protein (LOC10418) SEQ ID NOS: 118 (DNA) and 246 (amino acid)	Consensus includes gb:AB018305.1 /DEF=Homo sapiens mRNA for KIAA0762 protein, partial cds. /FEA=mRNA /GEN=KIAA0762 /PROD=KIAA0762 protein /DB_XREF=gi:3882244 /UG=Hs.5378 spondin 1, (f-spondin) extracellular matrix protein /FL=gb:AB051390.1	209436_at
COL11A1: collagen, type XI, alpha 1 (LOC1301) SEQ ID NOS: 119 (DNA) and 247 (amino acid)	Cluster Incl. J04177:Human alpha-1 type XI collagen (COL11A1) mRNA, complete cds /cds=(161,5581) /gb=J04177 /gi=179729 /ug=Hs.82772 /len=6158	37892_at
MAFB: v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian) (LOC9935) SEQ ID NOS: 120 (DNA) and 248 (amino acid)	gb:NM_005461.1 /DEF=Homo sapiens Kreisler (mouse) maf-related leucine zipper homolog (KRML), mRNA. /FEA=mRNA /GEN=KRML /PROD=Kreisler (mouse) maf-related leucine zipperhomolog /DB_XREF=gi:4885446 /UG=Hs.169487 Kreisler (mouse) maf-related leucine zipper homolog /FL=gb:AF134157.1 gb:NM_005461.1	218559_s_at
DDX17: DEAD (Asp-Glu-Ala-Asp) box polypeptide 17 (LOC10521) SEQ ID NOS: 121 (DNA) and 249 (amino acid)	Consensus includes gb:AW188131 /FEA=EST /DB_XREF=gi:6462567 /DB_XREF=est:xj92f11.x1 /CLONE=IMAGE:2664717 /UG=Hs.6179 DEADH (Asp-Glu-Ala-AspHis) box polypeptide 17 (72kD)	213998_s_at
PHLDA1: pleckstrin homology-like domain, family A, member 1 (LOC22822) SEQ ID NOS: 122 (DNA) and 250 (amino acid)	Consensus includes gb:NM_007350.1 /DEF=Homo sapiens pleckstrin homology-like domain, family A, member 1 (PHLDA1), mRNA. /FEA=mRNA /GEN=PHLDA1 /PROD=pleckstrin homology-like domain, family A,member 1 /DB_XREF=gi:6679302 /UG=Hs.82101 pleckstrin homology-like domain, family A, member 1 /FL=gb:NM_007350.1	217999_s_at
ETV5: ets variant gene 5 (ets-related molecule) (LOC2119) SEQ ID NOS: 123 (DNA) and 251 (amino acid)	gb:NM_004454.1 /DEF=Homo sapiens ets variant gene 5 (ets-related molecule) (ETV5), mRNA. /FEA=mRNA /GEN=ETV5 /PROD=ets variant gene 5 (ets-related	203349_s_at

	molecule) /DB_XREF=gi:4758315 /UG=Hs.43697 ets variant gene 5 (ets-related molecule) /FL=gb:NM_004454.1	
DUSP4: dual specificity phosphatase 4 (LOC1846) SEQ ID NOS: 124 (DNA) and 252 (amino acid)	gb:BC002671.1 /DEF=Homo sapiens, dual specificity phosphatase 4, clone MGC:3713, mRNA, complete cds. /FEA=mRNA /PROD=dual specificity phosphatase 4 /DB_XREF=gi:12803670 /UG=Hs.2359 dual specificity phosphatase 4 /FL=gb:U48807.1 gb:NM_001394.2 gb:BC002671.1 gb:U21108.1	204015_s_at
DUSP4: dual specificity phosphatase 4 (LOC1846) SEQ ID NOS: 125 (DNA) and 253 (amino acid)	gb:NM_001394.2 /DEF=Homo sapiens dual specificity phosphatase 4 (DUSP4), mRNA. /FEA=mRNA /GEN=DUSP4 /PROD=dual specificity phosphatase 4 /DB_XREF=gi:12707552 /UG=Hs.2359 dual specificity phosphatase 4 /FL=gb:U48807.1 gb:NM_001394.2 gb:BC002671.1 gb:U21108.1	204014_at
POFUT1: protein O-fucosyltransferase 1 (LOC23509) SEQ ID NOS: 126 (DNA) and 254 (amino acid)	Consensus includes gb:AL045513 /FEA=EST /DB_XREF=gi:5433649 /DB_XREF=est:DKFZp434J015_r1 /CLONE=DKFZp434J015 /UG=Hs.178292 KIAA0180 protein	212349_at
TBXAS1: thromboxane A synthase 1 (platelet, cytochrome P450, family 5, subfamily A) (LOC6916) SEQ ID NOS: 127 (DNA) and 255 (amino acid)	gb:NM_030984.1 /DEF=Homo sapiens thromboxane A synthase 1 (platelet, cytochrome P450, subfamily V) (TBXAS1), transcript variant TXS-II, mRNA. /FEA=mRNA /GEN=TBXAS1 /PROD=thromboxane A synthase 1 (platelet, cytochromeP450, subfamily V), isoform TXS-II /DB_XREF=gi:13699839 /FL=gb:NM_030984.1	208130_s_at
KCNK5: potassium channel, subfamily K, member 5 (LOC8645) SEQ ID NOS: 128 (DNA) and 256 (amino acid)	gb:NM_003740.1 /DEF=Homo sapiens potassium channel, subfamily K, member 5 (TASK-2) (KCNK5), mRNA. /FEA=mRNA /GEN=KCNK5 /PROD=potassium channel, subfamily K, member 5(TASK-2) /DB_XREF=gi:4504850	219615_s_at

	/UG=Hs.127007 potassium channel, subfamily K, member 5 (TASK-2) /FL=gb:AF084830.1 gb:NM_003740.1	

The biomarkers provided in Table 1, which include the nucleotide sequences of SEQ ID NOS:1-128 and the amino acid sequences of SEQ ID NOS:129-256, are referred to herein as a total of 128 biomarkers with reference to the Unigene Title.

The biomarkers have expression levels in cells that may be dependent on the activity of the EGFR signal transduction pathway, and that are also highly correlated with EGFR modulator sensitivity exhibited by the cells. Biomarkers serve as useful molecular tools for predicting the likelihood of a response to EGFR modulators, preferably biological molecules, small molecules, and the like that affect EGFR kinase activity via direct or indirect inhibition or antagonism of EGFR kinase function or activity.

WILD TYPE K-RAS AND MUTATED K-RAS

As used herein, wild type K-Ras can be selected from the K-Ras variant a and variant b nucleotide and amino acid sequences. Wild type K-Ras variant a has a nucleotide sequence that is 5436 nucleotides (GenBank Accession No. NM_033360.2) and encodes a protein that is 189 amino acids (GenBank Accession No. NP_203524.1) . Wild type K-Ras variant b has a nucleotide sequence that is 5312 nucleotides (GenBank Accession No. NM_004985.3) and encodes a protein that is 188 amino acids (GenBank Accession No. NP_004976.2).

A mutated form of K-Ras is a nucleotide or amino acid sequence that differs from wild type K-Ras at least at one position, preferably at least one nucleotide position that encodes an amino acid that differs from wild type K-Ras. In one aspect, the mutated form of K-Ras includes at least one mutation in exon 2. In another aspect, the mutated form of K-RAS includes at least one of the following mutations in exon 2 (base change (amino acid change)): 200G>A (V7M); 216G>C (G12A); 215G>T (G12C); 216G>A (G12D); 215G>C (G12R); 215G>A (G12S); 216G>T (G12V); 218G>T (G13C); 219G>A (G13D).

Methods for detecting K-Ras mutations are well known in the art and include, for example, the methods described in PCT Publication No. Wo2005/118876.

EGFR MODULATORS

As used herein, the term "EGFR modulator" is intended to mean a compound or drug that is a biological molecule or a small molecule that directly or indirectly modulates EGFR activity or the EGFR signal transduction pathway. Thus, compounds or drugs as used herein is intended to include both small molecules and biological molecules. Direct or indirect modulation includes activation or inhibition of EGFR activity or the EGFR signal transduction pathway. In one aspect, inhibition refers to inhibition of the binding of EGFR to an EGFR ligand such as, for example, EGF. In another aspect, inhibition refers to inhibition of the kinase activity of EGFR.

EGFR modulators include, for example, EGFR-specific ligands, small molecule EGFR inhibitors, and EGFR monoclonal antibodies. In one aspect, the EGFR modulator inhibits EGFR activity and/or inhibits the EGFR signal transduction pathway. In another aspect, the EGFR modulator is an EGFR monoclonal antibody that inhibits EGFR activity and/or inhibits the EGFR signal transduction pathway.

EGFR modulators include biological molecules or small molecules. Biological molecules include all lipids and polymers of monosaccharides, amino acids, and nucleotides having a molecular weight greater than 450. Thus, biological molecules include, for example, oligosaccharides and polysaccharides; oligopeptides, polypeptides, peptides, and proteins; and oligonucleotides and polynucleotides. Oligonucleotides and polynucleotides include, for example, DNA and RNA.

Biological molecules further include derivatives of any of the molecules described above. For example, derivatives of biological molecules include lipid and glycosylation derivatives of oligopeptides, polypeptides, peptides, and proteins.

Derivatives of biological molecules further include lipid derivatives of oligosaccharides and polysaccharides, e.g., lipopolysaccharides. Most typically, biological molecules are antibodies, or functional equivalents of antibodies. Functional equivalents of antibodies have binding characteristics comparable to those of antibodies, and inhibit the growth of cells that express EGFR. Such functional equivalents include, for example, chimerized, humanized, and single chain antibodies as well as fragments thereof.

Functional equivalents of antibodies also include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the antibodies. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions, additions, and/or deletions, is considered to be an equivalent sequence. Preferably, less than 50%, more preferably less than 25%, and still more preferably less than 10%, of the number of amino acid residues in a sequence are substituted for, added to, or deleted from the protein.

The functional equivalent of an antibody is preferably a chimerized or humanized antibody. A chimerized antibody comprises the variable region of a non-human antibody and the constant region of a human antibody. A humanized antibody comprises the hypervariable region (CDRs) of a non-human antibody. The variable region other than the hypervariable region, e.g., the framework variable region, and the constant region of a humanized antibody are those of a human antibody.

Suitable variable and hypervariable regions of non-human antibodies may be derived from antibodies produced by any non-human mammal in which monoclonal antibodies are made. Suitable examples of mammals other than humans include, for example, rabbits, rats, mice, horses, goats, or primates.

Functional equivalents further include fragments of antibodies that have binding characteristics that are the same as, or are comparable to, those of the whole antibody. Suitable fragments of the antibody include any fragment that comprises a sufficient portion of the hypervariable (i.e., complementarity determining) region to bind specifically, and with sufficient affinity, to EGFR tyrosine kinase to inhibit growth of cells that express such receptors.

Such fragments may, for example, contain one or both Fab fragments or the $F(ab')_2$ fragment. Preferably, the antibody fragments contain all six complementarity determining regions of the whole antibody, although functional fragments containing fewer than all of such regions, such as three, four, or five CDRs, are also included.

In one aspect, the fragments are single chain antibodies, or Fv fragments. Single chain antibodies are polypeptides that comprise at least the variable region of the heavy chain of the antibody linked to the variable region of the light chain, with or

without an interconnecting linker. Thus, Fv fragment comprises the entire antibody combining site. These chains may be produced in bacteria or in eukaryotic cells.

The antibodies and functional equivalents may be members of any class of immunoglobulins, such as IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof. In one aspect, the antibodies are members of the IgG1 subclass. The functional equivalents may also be equivalents of combinations of any of the above classes and subclasses.

In one aspect, EGFR antibodies can be selected from chimerized, humanized, fully human, and single chain antibodies derived from the murine antibody 225 described in U.S. Patent No. 4,943,533.

In another aspect, the EGFR antibody is cetuximab (IMC-C225) which is a chimeric (human/mouse) IgG monoclonal antibody, also known under the tradename ERBITUX. Cetuximab Fab contains the Fab fragment of cetuximab, i.e., the heavy and light chain variable region sequences of murine antibody M225 (U.S. Application No. 2004/0006212, incorporated herein by reference) with human IgG1 C_H1 heavy and kappa light chain constant domains. Cetuximab includes all three IgG1 heavy chain constant domains.

In another aspect, the EGFR antibody can be selected from the antibodies described in U.S. Patent No. 6,235,883, U.S. Patent No. 5,558,864, and U.S. Patent No. 5,891,996. The EGFR antibody can be, for example, AGX-EGF (Amgen Inc.) (also known as panitumumab) which is a fully human IgG2 monoclonal antibody. The sequence and characterization of ABX-EGF, which was formerly known as clone E7.6.3, is disclosed in U.S. Patent No. 6,235,883 at column 28, line 62 through column 29, line 36 and Figures 29-34, which is incorporated by reference herein. The EGFR antibody can also be, for example, EMD72000 (Merck KGaA), which is a humanized version of the murine EGFR antibody EMD 55900. The EGFR antibody can also be, for example: h-R3 (TheraCIM), which is a humanized EGFR monoclonal antibody; Y10 which is a murine monoclonal antibody raised against a murine homologue of the human EGFRvIII mutation; or MDX-447 (Medarex Inc.).

In addition to the biological molecules discussed above, the EGFR modulators useful in the invention may also be small molecules. Any molecule that is not a biological molecule is considered herein to be a small molecule. Some examples of

small molecules include organic compounds, organometallic compounds, salts of organic and organometallic compounds, saccharides, amino acids, and nucleotides. Small molecules further include molecules that would otherwise be considered biological molecules, except their molecular weight is not greater than 450. Thus, small molecules may be lipids, oligosaccharides, oligopeptides, and oligonucleotides and their derivatives, having a molecular weight of 450 or less.

It is emphasized that small molecules can have any molecular weight. They are merely called small molecules because they typically have molecular weights less than 450. Small molecules include compounds that are found in nature as well as synthetic compounds. In one embodiment, the EGFR modulator is a small molecule that inhibits the growth of tumor cells that express EGFR. In another embodiment, the EGFR modulator is a small molecule that inhibits the growth of refractory tumor cells that express EGFR.

Numerous small molecules have been described as being useful to inhibit EGFR.

One example of a small molecule EGFR antagonist is IRESSA (ZD1939), which is a quinozaline derivative that functions as an ATP-mimetic to inhibit EGFR. See, U.S. Patent No. 5,616,582; WO 96/33980 at page 4. Another example of a small molecule EGFR antagonist is TARCEVA (OSI-774), which is a 4-(substitutedphenylamino)quinozaline derivative [6,7-Bis(2-methoxy-ethoxy)-quinazolin-4-yl]-(3-ethynyl-l-phenyl)amine hydrochloride] EGFR inhibitor. See WO 96/30347 (Pfizer Inc.) at, for example, page 2, line 12 through page 4, line 34 and page 19, lines 14-17. TARCEVA may function by inhibiting phosphorylation of EGFR and its downstream PI3/Akt and MAP (mitogen activated protein) kinase signal transduction pathways resulting in p27-mediated cell-cycle arrest. See Hidalgo et al., Abstract 281 presented at the 37th Annual Meeting of ASCO, San Francisco, CA, 12-15 May 2001.

Other small molecules are also reported to inhibit EGFR, many of which are thought to be specific to the tyrosine kinase domain of an EGFR. Some examples of such small molecule EGFR antagonists are described in WO 91/116051, WO96/30347, WO96/33980, WO97/27199. WO97/30034, WO97/42187, WO97/49688, WO98/33798, WO00/18761, and WO00/31048. Examples of specific small molecule EGFR antagonists include C1-1033 (Pfizer Inc.), which is a quinozaline (N-[4-(3-

chloro-4-fluoro-phenylamino)-7-(3-mprpholin-4-yl-propoxy)-quinazolin-6-yl]-acrylamide) inhibitor of tyrosine kinases, particularly EGFR and is described in WO00/31048 at page 8, lines 22-6; PKI166 (Novartis), which is a pyrrolopyrimidine inhibitor of EGFR and is described in WO97/27199 at pages 10-12; GW2016 (GlaxoSmitbKline), which is an inhibitor of EGFR and HER2; EKB569 (Wyeth), which is reported to inhibit the growth of tumor cells that overexpress EGFR or HER2 in vitro and in vivo; AG-1478 (Tryphostin), which is a quinazoline small molecule that inhibits signaling from both EGFR and erbB-2; AG-1478 (Sugen), which is a bisubstrate inhibitor that also inhibits protein kinase CK2; PD 153035 (Parke-Davis) which is reported to inhibit EGFR kinase activity and tumor growth, induce apoptosis in cells in culture, and enhance the cytotoxicity of cytotoxic chemotherapeutic agents; SPM-924 (Schwarz Pharma), which is a tyrosine kinase inhibitor targeted for treatment of prostrate cancer; CP-546,989 (OSI Pharmaceuticals), which is reportedly an inhibitor of angiogenesis for treatment of solid tumors; ADL-681, which is a EGFR kinase inhibitor targeted for treatment of cancer; PD 158780, which is a pyridopyrimidine that is reported to inhibit the tumor growth rate ofA4431 xenografts in mice; CP-358,774, which is a quinzoline that is reported to inhibit autophosphorylation in HN5 xenografts in mice; ZD1839, which is a quinzoline that is reported to have antitumor activity in mouse xenograft models including vulvar, NSCLC, prostrate, ovarian, and colorectal cancers; CGP 59326A, which is a pyrrolopyrimidine that is reported to inhibit growth of EGFR-positive xenografts in mice; PD 165557 (Pfizer); CGP54211 and CGP53353 (Novartis), which are dianilnophthalimides. Naturally derived EGFR tyrosine kinase inhibitors include genistein, herbimycin A, quercetin, and erbstatin.

Further small molecules reported to inhibit EGFR and that are therefore within the scope of the present invention are tricyclic compounds such as the compounds described in U.S. Patent No. 5,679,683; quinazoline derivatives such as the derivatives described in U.S. Patent No. 5,616,582; and indole compounds such as the compounds described in U.S. Patent No. 5,196,446.

Further small molecules reported to inhibit EGFR and that are therefore within the scope of the present invention are styryl substituted heteroaryl compounds such as the compounds described in U.S. Patent No. 5,656,655. The heteroaryl group is a

monocyclic ring with one or two heteroatoms, or a bicyclic ring with 1 to about 4 heteroatoms, the compound being optionally substituted or polysubstituted.

Further small molecules reported to inhibit EGFR and that are therefore within the scope of the present invention are bis mono and/or bicyclic aryl heteroaryl, carbocyclic, and heterocarbocyclic compounds described in U.S. Patent No. 5,646,153.

Further small molecules reported to inhibit EGFR and that are therefore within the scope of the present invention is the compound provided Figure 1 of Fry et al., Science 265, 1093-1095 (1994) that inhibits EGFR.

Further small molecules reported to inhibit EGFR and that are therefore within the scope of the present invention are tyrphostins that inhibit EGFR/HER1 and HER 2, particularly those in Tables I, II, III, and IV described in Osherov et al., J. Biol. Chem., 25;268(15):11134-42 (1993).

Further small molecules reported to inhibit EGFR and that are therefore within the scope of the present invention is a compound identified as PD166285 that inhibits the EGFR, PDGFR, and FGFR families of receptors. PD166285 is identified as 6-(2,6-dichlorophenyl)-2-(4-(2-diethylaminoethyoxy)phenylamino)-8-methyl-8H-pyrido(2,3-d)pyrimidin-7-one having the structure shown in Figure 1 on page 1436 of Panek et al., Journal of Pharmacology and Experimental Therapeutics 283, 1433-1444 (1997).

It should be appreciated that useful small molecule to be used in the invention are inhibitors of EGFR, but need not be completely specific for EGFR.

BIOMARKERS AND BIOMARKER SETS

The invention includes individual biomarkers and biomarker sets having both diagnostic and prognostic value in disease areas in which signaling through EGFR or the EGFR pathway is of importance, e.g., in cancers or tumors, in immunological disorders, conditions or dysfunctions, or in disease states in which cell signaling and/or cellular proliferation controls are abnormal or aberrant. The biomarker sets comprise a plurality of biomarkers such as, for example, a plurality of the biomarkers provided in Table 1, that highly correlate with resistance or sensitivity to one or more EGFR modulators.

The biomarkers and biomarker sets of the invention enable one to predict or reasonably foretell the likely effect of one or more EGFR modulators in different biological systems or for cellular responses. The biomarkers and biomarker sets can be used in in vitro assays of EGFR modulator response by test cells to predict in vivo outcome. In accordance with the invention, the various biomarkers and biomarker sets described herein, or the combination of these biomarker sets with other biomarkers or markers, can be used, for example, to predict how patients with cancer might respond to therapeutic intervention with one or more EGFR modulators.

A biomarker and biomarker set of cellular gene expression patterns correlating with sensitivity or resistance of cells following exposure of the cells to one or more EGFR modulators provides a useful tool for screening one or more tumor samples before treatment with the EGFR modulator. The screening allows a prediction of cells of a tumor sample exposed to one or more EGFR modulators, based on the expression results of the biomarker and biomarker set, as to whether or not the tumor, and hence a patient harboring the tumor, will or will not respond to treatment with the EGFR modulator.

The biomarker or biomarker set can also be used as described herein for monitoring the progress of disease treatment or therapy in those patients undergoing treatment for a disease involving an EGFR modulator.

The biomarkers also serve as targets for the development of therapies for disease treatment. Such targets may be particularly applicable to treatment of colorectal cancer. Indeed, because these biomarkers are differentially expressed in sensitive and resistant cells, their expression patterns are correlated with relative intrinsic sensitivity of cells to treatment with EGFR modulators. Accordingly, the biomarkers highly expressed in resistant cells may serve as targets for the development of new therapies for the tumors which are resistant to EGFR modulators, particularly EGFR inhibitors.

The level of biomarker protein and/or mRNA can be determined using methods well known to those skilled in the art. For example, quantification of protein can be carried out using methods such as ELISA, 2-dimensional SDS PAGE, Western blot, immunopreciptation, immunohistochemistry, fluorescence activated cell sorting (FACS), or flow cytometry. Quantification of mRNA can be carried out using

methods such as PCR, array hybridization, Northern blot, in-situ hybridization, dot-blot, Taqman, or RNAse protection assay.

MICROARRAYS

The invention also includes specialized microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, comprising one or more biomarkers, showing expression profiles that correlate with either sensitivity or resistance to one or more EGFR modulators. Such microarrays can be employed in *in vitro* assays for assessing the expression level of the biomarkers in the test cells from tumor biopsies, and determining whether these test cells are likely to be resistant or sensitive to EGFR modulators. For example, a specialized microarray can be prepared using all the biomarkers, or subsets thereof, as described herein and shown in Table 1. Cells from a tissue or organ biopsy can be isolated and exposed to one or more of the EGFR modulators. In one aspect, following application of nucleic acids isolated from both untreated and treated cells to one or more of the specialized microarrays, the pattern of gene expression of the tested cells can be determined and compared with that of the biomarker pattern from the control panel of cells used to create the biomarker set on the microarray. Based upon the gene expression pattern results from the cells that underwent testing, it can be determined if the cells show a resistant or a sensitive profile of gene expression. Whether or not the tested cells from a tissue or organ biopsy will respond to one or more of the EGFR modulators and the course of treatment or therapy can then be determined or evaluated based on the information gleaned from the results of the specialized microarray analysis.

ANTIBODIES

The invention also includes antibodies, including polyclonal or monoclonal, directed against one or more of the polypeptide biomarkers. Such antibodies can be used in a variety of ways, for example, to purify, detect, and target the biomarkers of the invention, including both in vitro and in vivo diagnostic, detection, screening, and/or therapeutic methods.

KITS

The invention also includes kits for determining or predicting whether a patient would be susceptible or resistant to a treatment that comprises one or more EGFR modulators. The patient may have a cancer or tumor such as, for example, colorectal cancer. Such kits would be useful in a clinical setting for use in testing a patient's biopsied tumor or other cancer samples, for example, to determine or predict if the patient's tumor or cancer will be resistant or sensitive to a given treatment or therapy with an EGFR modulator. The kit comprises a suitable container that comprises: one or more microarrays, e.g., oligonucleotide microarrays or cDNA microarrays, that comprise those biomarkers that correlate with resistance and sensitivity to EGFR modulators, particularly EGFR inhibitors; one or more EGFR modulators for use in testing cells from patient tissue specimens or patient samples; and instructions for use. In addition, kits contemplated by the invention can further include, for example, reagents or materials for monitoring the expression of biomarkers of the invention at the level of mRNA or protein, using other techniques and systems practiced in the art such as, for example, RT-PCR assays, which employ primers designed on the basis of one or more of the biomarkers described herein, immunoassays, such as enzyme linked immunosorbent assays (ELISAs), immunoblotting, e.g., Western blots, or in situ hybridization, and the like.

APPLICATION OF BIOMARKERS AND BIOMARKER SETS

The biomarkers and biomarker sets may be used in different applications. Biomarker sets can be built from any combination of biomarkers listed in Table 1 to make predictions about the effect of an EGFR modulator in different biological systems. The various biomarkers and biomarkers sets described herein can be used, for example, as diagnostic or prognostic indicators in disease management, to predict how patients with cancer might respond to therapeutic intervention with compounds that modulate the EGFR, and to predict how patients might respond to therapeutic intervention that modulates signaling through the entire EGFR regulatory pathway.

The biomarkers have both diagnostic and prognostic value in diseases areas in which signaling through EGFR or the EGFR pathway is of importance, e.g., in

immunology, or in cancers or tumors in which cell signaling and/or proliferation controls have gone awry.

In one aspect, cells from a patient tissue sample, e.g., a tumor or cancer biopsy, can be assayed to determine the expression pattern of one or more biomarkers prior to treatment with one or more EGFR modulators. In one aspect, the tumor or cancer is colorectal. Success or failure of a treatment can be determined based on the biomarker expression pattern of the cells from the test tissue (test cells), e.g., tumor or cancer biopsy, as being relatively similar or different from the expression pattern of a control set of the one or more biomarkers. Thus, if the test cells show a biomarker expression profile which corresponds to that of the biomarkers in the control panel of cells which are sensitive to the EGFR modulator, it is highly likely or predicted that the individual's cancer or tumor will respond favorably to treatment with the EGFR modulator. By contrast, if the test cells show a biomarker expression pattern corresponding to that of the biomarkers of the control panel of cells which are resistant to the EGFR modulator, it is highly likely or predicted that the individual's cancer or tumor will not respond to treatment with the EGFR modulator.

The invention also provides a method of monitoring the treatment of a patient having a disease treatable by one or more EGFR modulators. The isolated test cells from the patient's tissue sample, e.g., a tumor biopsy or tumor sample, can be assayed to determine the expression pattern of one or more biomarkers before and after exposure to an EGFR modulator wherein, preferably, the EGFR modulator is an EGFR inhibitor. The resulting biomarker expression profile of the test cells before and after treatment is compared with that of one or more biomarkers as described and shown herein to be highly expressed in the control panel of cells that are either resistant or sensitive to an EGFR modulator. Thus, if a patient's response is sensitive to treatment by an EGFR modulator, based on correlation of the expression profile of the one or biomarkers, the patient's treatment prognosis can be qualified as favorable and treatment can continue. Also, if, after treatment with an EGFR modulator, the test cells don't show a change in the biomarker expression profile corresponding to the control panel of cells that are sensitive to the EGFR modulator, it can serve as an indicator that the current treatment should be modified, changed, or even discontinued. This monitoring process can indicate success or failure of a patient's

treatment with an EGFR modulator and such monitoring processes can be repeated as necessary or desired.

EXAMPLES:

EXAMPLE 1 - Interim Analysis Identification of Biomarkers

The CA225-045 pharmacogenomics trial is a phase II randomized exploratory study of ERBITUX (cetuximab) monotherapy in patients with refractory metastatic colorectal cancer (mCRC). An interim analysis of data from samples obtained from this trial was performed to examine the preclinically discovered markers in the clinical samples and to identify response prediction markers de novo.

Clinical samples:

49 RNA patient samples isolated from pre-treatment tumor biopsies of the metastatic site were randomized into five blocks and profiled on U133A v2.0 chips (Affymetrix, Santa Clara, California). Profiling data from 30/49 patients were included in the analysis based on meeting the following criteria: completion of at least two cycles of therapy; availability of sufficient clinical data to evaluate response; presence of tumor cells in biopsy sample; and good quality profiling data from chip.

The 30 patient expression profiles consisted of 24 liver metastases and 6 other tissue types. The Best Clinical Response information from the 30 patients identified 4 partial responders (PR), 5 stable disease (SD) and 21 progressive disease (PD) patients. Assessment of response was performed according to a modified version of the World Health Organization (WHO) criteria (Miller et al., Cancer, 47: 207-214 (1981)). Overall response was determined based on evaluation of target, non-target, and new lesions. Partial response (PR) was defined as at least a 50% decrease in the sum of the product of diameters (SPD) of target lesions, taking as reference the baseline SPD. Progressive disease (PD) was defined as a 25% or greater increase in the SPD of target lesions, taking as reference the smallest SPD recorded since the treatment started or the appearance of new lesions. Stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

Gene expression profiling:

Pre-treatment biopsies were obtained from the metastatic site for RNA isolation. RNA was isolated from the pre-treatment biopsies using the RNeasy mini kit (Qiagen, Valencia, California). The quality of RNA was checked by measuring the 28S:18S ribosomal RNA ratio using an Agilent 2100 Bioanalyzer (Agilent Technologies, Rockville, Maryland). Concentration of total RNA was determined spectrophotometrically. 1 μg of total RNA was used to prepare biotinylated probes according to the Affymetrix Genechip Expression Analysis Technical Manual. Targets were hybridized to human HG-U133A v2.0 gene chips according to the manufacturer's instructions. Data were preprocessed using the MAS 5.0 software (Affymetrix, Santa Clara, California).

Data Analysis:

Of the 22,215 probesets present on the U133A v2.0 chip, 17,261 probesets that had present calls in at least two liver metastatic tissues were included for data analysis. Data was analyzed by performing a two-sided unequal variance t test with Microsoft Excel or Anova analysis using PartekPro Pattern Recognition Software (Partek, St. Charles, Missouri). The statistical analyses were performed using MAS 5.0 quantile normalized values for signal intensity for 17,261 probe sets.

Analysis of biomarkers using t test and ANOVA analysis:

The first step was to examine 42 probesets that were identified preclinically (FIG. 1) in the transcriptional profiles of 30 metastatic tumors. This was done to examine whether the preclinical markers are differentially expressed between patients who derive clinical benefit (PR and SD) from ERBITUX treatment and those who do not (PD).

A two-sided unequal variance t test was performed between the 9 patients who derive clinical benefit and the 21 patients who have progressive disease. Three probesets out of 42 are differentially expressed between 9 (PR+SD) patients and 21 (PD) patients ($p<0.05$). These probesets represent the mRNA expression of Annexin A1 (*ANXA1* 201012_at), serine proteinase inhibitor clade B member 5 (*SERPINB5* 204855_at), and fibroblast growth factor receptor 3 (*FGFR3* 204379_s_at).

Next, a broader list of 640 genes from which the 42 probe set list had been derived (FIG. 1) was examined. 635 out of the 640 probesets were present in the 17,261 probe sets that are included in the analysis. The 635 probesets were identified as being highly variably expressed in transcriptional profiles of 164 primary untreated CRC tumors. They expressed at a moderate to high level in colon tumors (at least one expression value of two times the mean value for the array, i.e., 3000 expression units) and with a population variance value of >0.1.

The 635 probe sets were examined in transcriptional profiles of 30 metastatic tumors from the CA225-045 trial. 39 out of 635 probesets were found to be differentially expressed between 9 (PR+SD) and 21 (PD), $p<0.05$ and are described in Table 2. 19 of the 39 probe sets are resistance markers for ERBITUX and 20 of these are sensitivity markers for ERBITUX (FIG. 2).

TABLE 2 - 39 Markers for Response Prediction to ERBITUX

	Affymetrix ID	p value	Gene name	Symbol
1	205767_at	0.0002	epiregulin	EREG
2	201012_at	0.006	annexin A1	ANXA1
3	205239_at	0.0068	amphiregulin	AREG
4	213435_at	0.0098	SATB family member 2	SATB2
5	209260_at	0.0122	stratifin	SFN
6	204379_s_at	0.0129	fibroblast growth factor receptor 3	FGFR3
7	205295_at	0.0143	creatine kinase, mitochondrial 2	CKMT2
8	204678_s_at	0.0148	potassium channel, subfamily K, memb.1	KCNK1
9	204044_at	0.0151	quinolinate phosphoribosyltransferase	QPRT
10	203726_s_at	0.0154	laminin, alpha 3	LAMA3
11	219555_s_at	0.0165	uncharacterized bone marrow prtn BM039	BM039
12	216598_s_at	0.0188	chemokine (C-C motif) ligand 2	CCL2
13	209425_at	0.0195	alpha-methylacyl-CoA racemase	AMACR
14	204855_at	0.0207	serine proteinase inhibitor, clade B, memb. 5	SERPINB 5
15	218807_at	0.0213	vav 3 oncogene	VAV3
16	210764_s_at	0.0261	cysteine-rich, angiogenic inducer, 61	CYR61
17	210511_s_at	0.0265	inhibin, beta A	INHBA
18	220834_at	0.0266	membrane-spanning 4-domains, subfly A, 12	MS4A12
19	210809_s_at	0.0268	periostin, osteoblast specific factor	POSTN
20	213385_at	0.0304	chimerin 2	CHN2

21	218468_s_at	0.0323	gremlin 1 homolog, cysteine knot superfamily	GREM1
22	202859_x_at	0.033	interleukin 8	IL8
23	206754_s_at	0.0337	cytochrome P450, 2B6	CYP2B6
24	218806_s_at	0.034	vav 3 oncogene	VAV3
25	218469_at	0.0342	gremlin 1 homolog, cysteine knot superfamily	GREM1
26	219508_at	0.0347	glucosaminyl (N-acetyl) transferase 3, mucin type	GCNT3
27	204364_s_at	0.0367	chromosome 2 open reading frame 23	C2orf23
28	219471_at	0.0376	chromosome 13 open reading frame 18	C13orf18
29	219014_at	0.0396	placenta-specific 8	PLAC8
30	203939_at	0.04	5'-nucleotidase, ecto (CD73)	NT5E
31	211506_s_at	0.0401	interleukin 8	IL8
32	206143_at	0.0404	solute carrier family 26, member 3	SLC26A3
33	44790_s_at	0.0425	chromosome 13 open reading frame 18	C13orf18
34	202075_s_at	0.0427	phospholipid transfer protein	PLTP
35	201650_at	0.0436	keratin 19	KRT19
36	205259_at	0.046	nuclear receptor subfamily 3, C2	NR3C2
37	208893_s_at	0.0466	dual specificity phosphatase 6	DUSP6
38	209436_at	0.048	spondin 1, extracellular matrix protein	SPON1
39	218087_s_at	0.0496	sorbin and SH3 domain containing 1	SORBS1

The top 3 markers based on lowest p value were epiregulin (*EREG*, 205767_at), annexin A1 (*ANXA1* 201012_at), and amphiregulin (*AREG*, 205239_at). Interestingly, epiregulin and amphiregulin are ligands for EGFR. Examination of their individual mRNA expression profiles indicates that they appear to be more highly expressed in patients who derive clinical benefit from ERBITUX treatment (FIGS. 3A and 3B). This suggests that patients who have high levels of epiregulin and amphiregulin have tumors that are addicted to the EGFR signaling pathway that is being driven by these two ligands.

The expression levels of epidermal growth factor (EGF, 206254_at), transforming growth factor alpha (TGFα, 205016_at), betacellulin (BTC, 207326_at), and heparin binding-EGF (HB-EGF, 203821_ at), which are the other known ligands for EGFR, were also examined. Their expression levels showed no correlation with response to ERBITUX.

Determination of biological relationships between 39 biomarkers:

The Ingenuity Pathway Analysis web-based application (Ingenuity Systems Inc., Mountain View, California) was used to test the biological relationship between the 39 biomarkers of Table 2. This application makes use of the Ingenuity Knowledge Base, a curated database consisting of millions of individually modeled relationships between proteins, genes, complexes, cells, tissues, drugs, and diseases. The 39 genes were inputted into the Pathway Analysis application. The Ingenuity Knowledge base had information on 25 of the 39 genes. Strikingly, of the 25 "network eligible" genes, 17 mapped to the EGFR network (FIG. 4, 17 genes are shaded) indicating a strong link between the EGFR signaling status in the tumors and response to ERBITUX. No other network emerged from the analysis of the 39 genes. Of the 17 genes, DUSP6 is a member of the ERK/MAPK signaling pathway and SFN is a member of the PI3K/AKT signaling pathway, which are the two key pathways downstream of EGFR signaling.

Multivariate analysis:

The t test and ANOVA analysis was used to assess the ability of individual biomarkers to separate PR/SD patients from PD patients. Multivariate discriminant analysis was used to assess the prediction power of the 39 markers on patient response, and identify the set of variables/biomarkers that would be the best predictors of response to ERBITUX treatment.

SAS discriminant function analysis (SAS Scientific Discovery Solutions, release 8.02, SAS Institute Inc., Cary, North Carolina) was applied to the data set of 39 markers. Discriminant function analysis was broken into a 2-step process: (1) testing the significance of a set of discriminant functions; and (2) using these functions to classify the sample objects to the corresponding response groups. The first step was accomplished by a SAS "stepwise" procedure using the forward variable selection method. The derived discriminant functions were passed on to the second SAS procedure, called the "discrim" procedure, for classification of the given samples.

Given the small sample size of 30 patients, the samples were not partitioned into separate training and test data sets. Instead a single data set was used, and the

leave-one-out cross-validation method was applied to test the prediction power of the identified biomarker predictors. A SAS cross-validation protocol was developed, which implemented leave-one-out cross-validation method in a SAS program, and was run on this data set to define the number of predictors that could be used for building the discriminant function models. This method allowed a comparison of a single biomarker model to multiple biomarker models (up to 15 biomarkers) (FIG. 5). The single gene predictor model was found to have 0.7037 prediction power as measured by AUC coverage (area under the Receiver Operating Characteristic (ROC) curve which shows the tradeoff between sensitivity and specificity). An area of 1 represents completely accurate prediction. When the number of predictors included in the model goes up to three biomarkers, the prediction power increases to 0.9. When the number of predictors included in the model exceeds three, there tends to be a decrease in prediction power. These results indicate that the best prediction power is achieved by building a discriminant function model with 3 out of the 39 biomarkers.

Correlation of the 39 biomarkers:

Ingenuity Pathway analysis suggested that at least 17 of the 39 biomarkers identified belong to a single interaction network. A correlation analysis using SAS "corr" procedure was applied to investigate the correlation of genes identified from the discriminant analysis. Table 3 shows an example of a correlation matrix of some of the top predictors selected by the SAS procedure. Some of the genes show very high correlation coefficient values which suggests they are highly correlated. For example, 205767_at (EREG) and 205239_at (AREG), or 205767_at (EREG) and 218807_at (VAV3), or 206754_s_at (CYP2B6) and 209260_at (SFN) were found to be highly correlated. The highly correlated genes could replace each other to explain a certain proportion of the variation between the groups of patients who derive clinical benefit and those that do not. These results show excellent agreement between the possible biological mechanism as elucidated by Ingenuity Pathway Analysis and literature, and the statistical prediction as determined by the SAS procedure.

TABLE 3 - Pearson Correlation Co-Efficients on 7 Most Frequent Probesets That Were Identified As Top Variables For Discriminant Analysis

Affymetrix ID	205767_at	201012_at	205239_at	206754_at	209260_at	205259_at	218807_at
205767_at	1	-0.28587	0.84089	-0.16409	-0.04261	-0.02338	0.64133
201012_at	-0.28587	1	-0.16652	-0.41722	0.31615	-0.45851	0.28141
205239_at	0.84089	-0.16652	1	-0.21894	0.07064	-0.19815	0.60752
206754_s_at	-0.16409	-0.41722	-0.21894	1	-0.47769	0.53511	-0.21663
209260_at	-0.04261	0.31615	0.07064	-0.47769	1	-0.26621	0.26204
205259_at	-0.02338	-0.45851	-0.19815	0.53511	-0.26621	1	-0.02668
218807_at	0.64133	-0.28141	0.60752	-0.21663	0.26204	-0.02668	1

Best prediction models:

The best prediction models were determined using the SAS stepwise procedure. 205767_at (EREG) was always picked first. This suggests that the expression of the EGFR ligand epiregulin can explain most of the variation that exists between the group of patients that are PR/SD and the group of patients who are PD. The second predictor aids in picking up the largest proportion of the unexplained variation from the first variable function (predictor) and so on. The misclassification rates of the best SAS selected models were:

Model	Error rate
205767_at (EREG)	0.2143
205767_at (EREG), 206754_s_at (CYP2B6)	0.127
205767_at (EREG), 206754_s_at (CYP2B6), 201650_at (KRT19)	0.1032
205767_at (EREG), 206754_s_at (CYP2B6), 201650_at (KRT19), 204678_at (KCNK1)	0.1032

Biomarkers were also selected based on their biological, functional, and co-regulation information, and the derived prediction functions were used to classify the 30 sample data set using the SAS "discrim" procedure. Using this approach, some optimal

combinations of biomarker variables and their corresponding misclassification rates were identified, such as:

Model	Error rate
205767_at (EREG), 206754_s_at (CYP2B6) 201650_at (KRT19)	0.1032
205767_at (EREG), 209260_at (SFN), 205259_at (NR3C2)	0.079
201012_at (ANXA1), 205239_at (AREG), 209260_at (SFN), 205259_at (NR3C2), 218807_at (VAV3)	0.07
209260_at (SFN), 218807_at (VAV3)	0.1270

EXAMPLE 2 - Identification of Biomarkers following Interim Analysis

As mentioned above, the CA225-045 pharmacogenomics trial is a phase II randomized exploratory study of ERBITUX (cetuximab) monotherapy in patients with refractory metastatic colorectal cancer (mCRC). This trial enrolled 111 patients. A standard cetuximab dosing regimen was followed for the first 3 weeks of therapy, thereafter patients were eligible for dose escalation every 3 weeks to a maximum dose of 400 mg/m^2 provided they had not experienced a > grade 2 skin rash. During the pre-treatment phase, all patients underwent a tumor biopsy procedure involving three passes with an 18-gauge needle of a single metastatic lesion. Two pre-treatment core needle biopsies were stored in a single tube of RNALater at room temperature and one core needle biopsy was formalin-fixed and embedded in paraffin for subsequent analyses. All subjects also underwent a pre-treatment blood draw. All specimens were obtained from patients with appropriate informed consent and IRB approval.

Tumor response was evaluated every nine weeks (one cycle of therapy) according to the modified World Health Organization criteria (Miller et al., Cancer, 47, 207-214 (1981)). Overall response was determined based on evaluation of target, non-target and new lesions. For this analysis, subjects experiencing a complete (CR) or partial response (PR), or stable disease (SD), were grouped as the disease control group; progressive disease (PD) and select unable to determine (UTD) subjects were grouped as non-responders. The UTD subjects that were included in the non-responder group for analysis were those that died prior to the response assessment. All other UTD subjects were excluded from the analysis.

RNA and DNA extraction:

For each subject's tumor sample, RNA and DNA were isolated from two pre-treatment core needle biopsies provided in a single tube of RNALater at room temperature within seven days from the date of the biopsy procedure. RNA was isolated using the RNeasy mini kit (Qiagen, Valencia, California). The quality of RNA was checked by measuring the 28S:18S ribosomal RNA ratio using an Agilent 2100 Bioanalyzer (Agilent Technologies, Rockville, Maryland). DNA was isolated from the flow-through collected during the RNA isolation procedure using the DNeasy mini kit (Qiagen). Concentration of RNA and DNA was determined spectrophotometrically.

Gene expression profiling and statistical analysis:

For each sample from which sufficient RNA was available, 1 μg of total RNA was used to prepare biotinylated probes according to the Affymetrix GeneChip Expression Analysis Technical Manual. Targets were hybridized to human HG-U133A v2.0 GeneChips according to the manufacturer's instructions. Data were preprocessed using the MAS 5.0 software (Affymetrix, Santa Clara, California) and statistical analyses were performed using quantile normalized values for signal intensity. Univariate analysis was done by using a two-sided unequal variance t-test. For multivariate analysis samples were randomly partitioned 50-50 into a training set and a test set. Top candidate predictors were selected from the training set using a t-test. This was followed by model construction using stepwise discriminant analysis (v8.2, SAS, Cary, North Carolina). Class prediction was assessed using 10-fold cross validation. The models developed from the training set were evaluated using a test set.

In addition to the profiling of RNA from the clinical study, an expression database of 164 primary colorectal tumors (Banerjea et al., Mol. Cancer, 3, 21 (2004)) was examined to identify potential predictive markers. Data from the 640 probe sets that passed the filtering steps described above in the results were then subjected to an unsupervised average linkage hierarchical clustering using CLUSTER and the results were displayed by using TREEVIEW.

RT-qPCR for gene expression analysis:

For each sample from which RNA was available, approximately 100 ng RNA was converted into cDNA by the random priming method using MultiScribe Reverse Transcriptase according to the manufacturer's instructions (TaqMan Reverse Transcription Reagents, Applied Biosystems Inc. ((ABI), Foster City, California). The resulting cDNA was measured on the ABI 7900HT Sequence Detection System using ABI Assay-on-Demand primer/probe sets directed against the amphiregulin (Hs00155832_m1) and epiregulin (Hs00154995_m1) genes. Relative expression levels were calculated using the ΔCt method in which average values of duplicate reactions were compared, with GAPDH (Hs001266705_g1) serving as the internal reference. In this experimental design, low ΔCt values correspond to high levels of expression.

Nucleotide sequence analysis:

Mutational analyses of EGFR, K-RAS, and BRAF were performed using available genomic DNAs isolated from tumor specimens. Primers used for EGFR exons 18-21, coding for the TK domain, were published previously (Lynch et al., N. Engl. J. Med., 350, 2129-2139 (2004)). The primers used to evaluate exon 2 of K-RAS and exon 15 of BRAF were as follows: K-RAS F 5'-TAAGGCCTGCTGAAAATGACTG-3' (SEQ ID NO:257) and K-RAS R 5'-TGGTCCTGCACCAGTAA TATGC-3' (SEQ ID NO:258); BRAF F 5'-TCATAATGCTTGCTCTGATAGGA-3' (SEQ ID NO:259) and BRAF R 5'-GGCCAAAAATTTAATCAGTGGA-3' (SEQ ID NO:260). PCR was performed using conditions as previously described (Chen et al., Hum. Mutat., 27, 427-435 (2006)). PCR fragments were cleaned with QIAquick PCR Purification Kit (Qiagen), sequenced on an ABI 3100A Capillary Genetic Analyzer (Applied Biosystems Inc.) and analyzed in both sense and antisense directions for the presence of heterozygous mutations. Analysis of the DNA sequence was performed using SEQUENCHER v4.2 (Gene Codes, Ann Arbor, Michigan) followed by visual analysis of each electropherogram by two independent reviewers. Appropriate positive and negative controls were included for each of the exons evaluated. Mutational analyses were done without knowledge of clinical outcome including tumor response.

RESULTS

Patients' characteristics and clinical outcome:

The primary objective of this study was to identify predictive markers of response to cetuximab therapy in CRC. Evaluable RNA and/or DNA and/or plasma samples were available for 103 out of 111 subjects. The objective response determination for these 103 subjects were: one complete response (CR), six partial response (PR), twenty-eight stable disease (SD), fifty-six progressive disease (PD), and twelve patients who died prior to their first radiographic assessment and are therefore unable to determine (UTD). Thirty-four percent of the subjects either responded or had disease stabilization whereas the remaining 66% were classified as non-responders.

Genomic analysis of tumor-derived RNAs:

In order to identify genes that were differentially expressed between the disease control and non-responder groups, gene expression profiling was carried out on RNA isolated from 95 pre-treatment biopsies. Seventy percent of the biopsies were taken from the liver metastatic tissue, and 30% of the biopsies were taken from non-hepatic tissue sites. 91 out of the 95 samples yielded > 500 ng RNA and were randomized into ten blocks and profiled on U133A v2.0 chips (Affymetrix). High quality transcriptional profiling data were obtained from 87 patients. Seven patients were excluded from further analysis either because they withdrew from the study prior to the first assessment, experienced hypersensitivity or withdrew their consent. Final data analysis was carried out using best clinical response assessments for the remaining 80 patients and expression profiles from these patients were included in the statistical analysis. These 80 patients included 1 CR, 5 PR, 19 SD, 43 PD, and 12 UTD.

An initial candidate set of genes was identified that were variably expressed in an independent set of 164 primary colorectal tumors by filtering transcriptional data from all 22,215 probe sets. This filtering yielded 640 probe sets that were expressed at a moderate to high level in colon tumors (at least one expression value of two times

the mean value for the array i.e. 3000 expression units) and with a population variance value of >0.1. It was proposed that these 640 probe sets that had a highly dynamic range of expression across a population of CRC tumors were most likely to yield markers that would be useful for patient selection. Unsupervised hierarchical clustering of the 640 probe sets across the 164 primary colon tumors showed that biologically interesting genes that might be predictive of response to cetuximab were preferentially expressed in a subset of colorectal tumors (FIG. 6). In FIG. 6, the 164 tumors were divided into 3 major classes (Class 1, 2 and 3). The 640 probe sets were divided into 5 clusters (labeled A through E). Cluster A, which contains cancer antigens such as CEACAM 6 and CD24, also contains the EGFR ligands EREG and AREG. Cluster A is most highly expressed in Class 1a, which represents approximately 25% of the 164 colorectal tumor specimens.

Out of 22,215 probe sets, data analysis was conducted on 17,137 probe sets that were found to be expressed in at least 10% of the liver metastases patient samples. 629 of the previously identified 640 probe sets were present in the 17,137 probe set list. Their gene expression profiles were examined in the data from 80 patients and were correlated with response assessments. 121 out of the 629 probe sets were found to be differentially expressed between 25 patients with disease control and 55 non-responders, $p<0.05$ (t test of the disease group (CR, PR, SD) vs. non-responders) as shown in Table 4.

TABLE 4 - 121 Probe Sets Differentially Expressed Between 25 patients with disease control and 55 non-responders, $p<0.05$

Affymetrix ID	p value	Gene name	Symbol
203939_at	3.787E-07	5'-nucleotidase, ecto (CD73)	NT5E
205767_at	1.474E-05	epiregulin	EREG
205239_at	2.489E-05	amphiregulin (schwannoma-derived growth factor)	AREG
213975_s_at	3.617E-05	lysozyme (renal amyloidosis) /// leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	LYZ /// LILRB1
201641_at	9.146E-05	bone marrow stromal cell antigen 2	BST2
208893_s_at	0.000257	dual specificity phosphatase 6	DUSP6
218807_at	0.000507	vav 3 oncogene	VAV3
218806_s_at	0.000513	vav 3 oncogene	VAV3

216598_s_at	0.000680	chemokine (C-C motif) ligand 2	CCL2
213435_at	0.000909	SATB family member 2	SATB2
210517_s_at	0.001636	A kinase (PRKA) anchor protein (gravin) 12	AKAP12
219508_at	0.001935	glucosaminyl (N-acetyl) transferase 3, mucin type	GCNT3
201462_at	0.001937	secernin 1	SCRN1
204379_s_at	0.002008	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	FGFR3
206584_at	0.002018	lymphocyte antigen 96	LY96
200884_at	0.002042	creatine kinase, brain	CKB
206332_s_at	0.002612	interferon, gamma-inducible protein 16	IFI16
202525_at	0.002630	protease, serine, 8 (prostasin)	PRSS8
205403_at	0.002869	interleukin 1 receptor, type II	IL1R2
221530_s_at	0.002881	basic helix-loop-helix domain containing, class B, 3	BHLHB3
209728_at	0.003260	major histocompatibility complex, class II, DR beta 4 /// major histocompatibility complex, class II, DR beta 4	HLA-DRB4
215049_x_at	0.004039	CD163 antigen	CD163
203645_s_at	0.004182	CD163 antigen	CD163
219471_at	0.004627	chromosome 13 open reading frame 18	C13orf18
210133_at	0.004790	chemokine (C-C motif) ligand 11	CCL11
205097_at	0.005553	solute carrier family 26 (sulfate transporter), member 2	SLC26A2
211656_x_at	0.006050	major histocompatibility complex, class II, DQ beta 1 /// major histocompatibility complex, class II, DQ beta 1	HLA-DQB1
209392_at	0.006150	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	ENPP2
205402_x_at	0.006181	protease, serine, 2 (trypsin 2)	PRSS2
217028_at	0.006582	chemokine (C-X-C motif) receptor 4	CXCR4
204855_at	0.006615	serpin peptidase inhibitor, clade B (ovalbumin), member 5	SERPINB5
201137_s_at	0.007369	major histocompatibility complex, class II, DP beta 1	HLA-DPB1
215051_x_at	0.007563	allograft inflammatory factor 1	AIF1
202859_x_at	0.007872	interleukin 8	IL8
211506_s_at	0.008119	interleukin 8	IL8
207457_s_at	0.008600	lymphocyte antigen 6 complex,	LY6G6D

		locus G6D	
205765_at	0.009101	cytochrome P450, family 3, subfamily A, polypeptide 5	CYP3A5
204619_s_at	0.009733	chondroitin sulfate proteoglycan 2 (versican)	CSPG2
205199_at	0.010621	carbonic anhydrase IX	CA9
219962_at	0.010751	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	ACE2
205242_at	0.011022	chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	CXCL13
217428_s_at	0.011274	collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)	COL10A1
206918_s_at	0.011540	copine I	CPNE1
44790_s_at	0.011645	chromosome 13 open reading frame 18	C13orf18
218469_at	0.011704	gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)	GREM1
209823_x_at	0.011862	major histocompatibility complex, class II, DQ beta 1	HLA-DQB1
205513_at	0.011867	transcobalamin I (vitamin B12 binding protein, R binder family)	TCN1
204213_at	0.012198	polymeric immunoglobulin receptor	PIGR
205941_s_at	0.012335	collagen, type X, alpha 1(Schmid metaphyseal chondrodysplasia)	COL10A1
212192_at	0.012522	potassium channel tetramerisation domain containing 12	KCTD12
204891_s_at	0.012755	lymphocyte-specific protein tyrosine kinase	LCK
208029_s_at	0.012800	lysosomal associated protein transmembrane 4 beta /// lysosomal associated protein transmembrane 4 beta	LAPTM4B
201884_at	0.013032	carcinoembryonic antigen-related cell adhesion molecule 5	CEACAM5
201030_x_at	0.013074	lactate dehydrogenase B	LDHB
202411_at	0.013302	interferon, alpha-inducible protein 27	IFI27
211165_x_at	0.013671	EPH receptor B2	EPHB2
212186_at	0.014902	acetyl-Coenzyme A carboxylase alpha	ACACA
201743_at	0.015156	CD14 antigen /// CD14 antigen	CD14
87100_at	0.015861	---	---
206467_x_at	0.015975	tumor necrosis factor receptor superfamily, member 6b, decoy /// regulator of telomere elongation helicase 1	TNFRSF6B /// RTEL1

218468_s_at	0.016329	gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)	GREM1
222257_s_at	0.016397	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	ACE2
221730_at	0.016992	collagen, type V, alpha 2	COL5A2
203915_at	0.017412	chemokine (C-X-C motif) ligand 9	CXCL9
206858_s_at	0.017492	homeo box C6	HOXC6
221584_s_at	0.017554	potassium large conductance calcium-activated channel, subfamily M, alpha member 1	KCNMA1
204475_at	0.018085	matrix metallopeptidase 1 (interstitial collagenase)	MMP1
203895_at	0.018353	phospholipase C, beta 4	PLCB4
214043_at	0.018926	Protein tyrosine phosphatase, receptor type, D	PTPRD
204678_s_at	0.019645	potassium channel, subfamily K, member 1	KCNK1
204446_s_at	0.019912	arachidonate 5-lipoxygenase	ALOX5
204533_at	0.020226	chemokine (C-X-C motif) ligand 10	CXCL10
211689_s_at	0.020262	transmembrane protease, serine 2 /// transmembrane protease, serine 2	TMPRSS2
201858_s_at	0.020471	proteoglycan 1, secretory granule	PRG1
212671_s_at	0.020852	major histocompatibility complex, class II, DQ alpha 1 /// major histocompatibility complex, class II, DQ alpha 2	HLA-DQA1 /// HLA-DQA2
216248_s_at	0.021062	nuclear receptor subfamily 4, group A, member 2	NR4A2
212188_at	0.021225	potassium channel tetramerisation domain containing 12 /// potassium channel tetramerisation domain containing 12	KCTD12
204070_at	0.021833	retinoic acid receptor responder (tazarotene induced) 3	RARRES3
213564_x_at	0.022061	lactate dehydrogenase B	LDHB
209732_at	0.022699	C-type lectin domain family 2, member B	CLEC2B
213746_s_at	0.023141	filamin A, alpha (actin binding protein 280)	FLNA
214974_x_at	0.023351	chemokine (C-X-C motif) ligand 5	CXCL5
201792_at	0.023592	AE binding protein 1	AEBP1
213905_x_at	0.023638	biglycan /// serologically defined colon cancer antigen 33	BGN /// SDCCAG33
212353_at	0.024175	sulfatase 1	SULF1
209156_s_at	0.024926	collagen, type VI, alpha 2	COL6A2

203083_at	0.025140	thrombospondin 2	THBS2
203896_s_at	0.025311	phospholipase C, beta 4	PLCB4
201617_x_at	0.025316	caldesmon 1	CALD1
217963_s_at	0.025667	nerve growth factor receptor (TNFRSF16) associated protein 1	NGFRAP1
208965_s_at	0.025706	interferon, gamma-inducible protein 16	IFI16
217763_s_at	0.026315	RAB31, member RAS oncogene family	RAB31
203325_s_at	0.026698	collagen, type V, alpha 1	COL5A1
209792_s_at	0.026893	kallikrein 10	KLK10
205549_at	0.027028	Purkinje cell protein 4	PCP4
204622_x_at	0.028026	nuclear receptor subfamily 4, group A, member 2	NR4A2
210095_s_at	0.030712	insulin-like growth factor binding protein 3	IGFBP3
209969_s_at	0.031010	signal transducer and activator of transcription 1, 91kDa	STAT1
202436_s_at	0.031792	cytochrome P450, family 1, subfamily B, polypeptide 1	CYP1B1
202311_s_at	0.032306	collagen, type I, alpha 1	COL1A1
221031_s_at	0.032415	hypothetical protein DKFZp434F0318 /// hypothetical protein DKFZp434F0318	DKFZP434F0318
209118_s_at	0.032949	tubulin, alpha 3	TUBA3
210164_at	0.033266	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) /// granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	GZMB
213194_at	0.034686	roundabout, axon guidance receptor, homolog 1 (Drosophila)	ROBO1
204697_s_at	0.034934	chromogranin A (parathyroid secretory protein 1)	CHGA
202752_x_at	0.035921	solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	SLC7A8
205929_at	0.037216	glycoprotein A33 (transmembrane)	GPA33
204044_at	0.037293	quinolinate phosphoribosyltransferase (nicotinate-nucleotide pyrophosphorylase (carboxylating))	QPRT
205311_at	0.037673	dopa decarboxylase (aromatic L-amino acid decarboxylase)	DDC
204320_at	0.038710	collagen, type XI, alpha 1	COL11A1
204364_s_at	0.040104	chromosome 2 open reading frame	C2orf23

		23	
212354_at	0.040347	sulfatase 1	SULF1
202465_at	0.040639	procollagen C-endopeptidase enhancer	PCOLCE
212992_at	0.041178	chromosome 14 open reading frame 78	C14orf78
209201_x_at	0.042126	chemokine (C-X-C motif) receptor 4	CXCR4
215646_s_at	0.043050	chondroitin sulfate proteoglycan 2 (versican) /// chondroitin sulfate proteoglycan 2 (versican)	CSPG2
202283_at	0.045795	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	SERPINF1
209436_at	0.046099	spondin 1, extracellular matrix protein	SPON1
37892_at	0.048675	collagen, type XI, alpha 1	COL11A1
218559_s_at	0.048679	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	MAFB
213998_s_at	0.049742	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	DDX17

The top three candidate markers based on lowest p value were 5'nucleotidase ecto (CD73, 203939_at), epiregulin (EREG, 205767_at) and amphiregulin (AREG, 205239_at). CD73 is a purine metabolizing enzyme that may have prognostic value in colorectal and pancreatic cancer (Eroglu et al., Med. Oncol., 17, 319-324 (2000); Giovannetti et al., Cancer Res., 66, 3928-3935 (2006)). Examination of its mRNA profile showed that it is expressed at higher levels in the non-responder group. Epiregulin and amphiregulin are ligands for EGFR (Singh and Harris, Cell Signal, 17, 1183-1193 (2005)). Examination of their individual mRNA expression profiles revealed that they were more highly expressed in patients in the disease control group (FIG. 7A and 7B). FIG. 7A and 7B provide mRNA levels of EGFR ligands epiregulin and amphiregulin. Affymetrix mRNA levels of Epiregulin (EREG, 205767_at) and Amphiregulin (AREG, 205239_at) are plotted on the y axis. There is a statistically significant difference in gene expression levels between the disease control group (CR, PR and SD) and the non-responder group (EREG $p = 1.474e^{-05}$, AREG $p = 2.489e^{-05}$). These results suggest that patients who have high levels of

EREG and AREG have tumors that are addicted to the EGFR signaling pathway and are therefore most likely to experience disease control on treatment with cetuximab.

In addition to the gene filtering approach described above, a de novo analysis was performed on the transcriptional profiles of the same 80 patients. A two-sided unequal-variance t-test was done on all 17,137 probe sets. The top 10 genes are provided in Table 5.

TABLE 5 - Top 10 Genes from De Novo Analysis

Affymetrix ID	p value	Gene name	Symbol
203939_at	3.787E-07	5'-nucleotidase, ecto (CD73)	NT5E
217999_s_at	7.056E-06	Pleckstrin homology-like domain, family A, member 1	PHLDA1
205767_at	1.474E-05	epiregulin	EREG
203349_s_at	1.704E-05	ets variant gene 5 (ets-related molecule)	ETV5
204015_s_at	1.812E-05	dual specificity phosphatase 4	DUSP4
204014_at	1.856E-05	dual specificity phosphatase 4	DUSP4
212349_at	2.395E-05	protein O-fucosyltransferase 1	POFUT1
205239_at	2.489E-05	amphiregulin (schwannoma-derived growth factor)	AREG
208130_s_at	2.646E-05	thromboxane A synthase 1 (platelet, cytochrome P450, family 5, subfamily A) /// thromboxane A synthase 1 (platelet, cytochrome P450, family 5, subfamily A)	TBXAS1
219615_s_at	3.153E-05	potassium channel, subfamily K, member 5	KCNK5

Examination of the top 10 genes with the lowest p value revealed that EREG and AREG were once again found to be top sensitivity markers. CD73, dual specificity phosphatase 4 (DUSP4, 204015_s_at and 204014_at), and pleckstrin homology like domain A1 (PHLDA1, 217999_s_at) were found to be top resistance markers. The mRNA expression levels of epidermal growth factor (EGF, 206254_at), transforming growth factor alpha (TGFα, 205016_at), betacellulin (BTC, 207326_at) and heparin binding –EGF (HB-EGF, 203821_at), some of the other known ligands for EGFR, were also reviewed. Their expression levels showed no correlation with response to

cetuximab. It is also worth noting that no correlation was seen between EGFR (201983_s_at) mRNA levels and response to cetuximab. These results suggest that a de novo analysis using only the transcriptional profiling data gathered from this clinical study could find the candidate markers EREG and AREG. However, given the issue of multiple test comparisons, the identification of EREG and AREG using an independent filtering approach described above lends additional support to their being candidates for predicting cetuximab response.

From the t-test analyses, the ability of individual biomarkers to separate the disease control group from the non-responders could be assessed. Using discriminant function analysis, the prediction power of a set of the 100 top candidate markers for patient response was assessed in order to identify the set of variables that would be the best predictors of disease control with cetuximab treatment. The AUC (area under the receiver operating characteristic curve) values of the different multi-gene models showed that as the number of genes in the model increased from one to fifteen the predictive power of the model did not improve. The AUC value of a single gene model was >0.8. An independent test was done to assess the performance of the most frequently identified gene, *EREG*, and also of *AREG*, as individual predictors. *EREG* has an AUC value of 0.845, and AREG has an AUC value of 0.815, indicating that they are both highly powerful predictive markers for patient selection (FIG. 8A and 8B).

Analysis of candidate markers epiregulin and amphiregulin:

In order to independently verify gene expression with a different technology platform that may ultimately be more easily transferable into a diagnostic test, AREG and EREG transcript levels were measured using quantitative RT-PCR TaqMan assays. Expression levels of these genes were obtained for tumor samples from 73 of the subjects using both array-based and qRT-PCR methods (Table 6).

TABLE 6 - Expression Levels of Amphiregulin and Epiregulin by quantitative RT-PCR TaqMan Assays

Best Clinical Response Assessment	AffyQ AREG expression	AffyQ EREG expression	qRT-PCR AREG dCt	qRT-PCR EREG dCt	KRAS Mutation codon base change	KRAS Mutation amino acid change	Order of sample on FIG. 7
CR	2573.74	1659.91	5.80	5.32			1
PD	949.81	450.25	7.79	7.20	WT		36
SD	3353.93	2336.8	9.58	8.89	c.35G>T	G12V	7
SD	105.82	89.23	9.35	9.31	WT		8
UTD	1581.54	603.27	6.48	6.20	c.35G>A	G12D	73
SD							
PD	1626.87	668.84	5.40	5.48	c.35G>T	G12V	32
PD	122.3	46.36					58
UTD	321.51	56.59	9.20	9.31	c.35G>A	G12D	69
SD							
SD							
PD	177.95	128.85	9.01	8.76	c.35G>A	G12D	67
PD	2550.49	655.04	4.57	5.64	WT		30
PR	3974.98	1108.91	3.23	4.38	WT		2
PD	1084.91	622.01	5.35	5.46	WT		26
PD	611.84	573.66	6.17	5.60	WT		47
SD	955.24	292.33	6.22	7.30	WT		11
PR	5083.12	1166.18			WT		5
PD							
SD	2481.22	1154.9	4.56	4.99	WT		12
SD	2527.86	1395.95	5.37	4.35	WT		13
SD					WT		
PD					c.35G>A	G12D	
PD	402.53	419.27	9.34	6.14	c.35G>A	G12D	62
PR	3395.09	1447.49	3.76	4.14	WT		3
PD	2134.23	906.03	7.11	6.45	c.35G>T	G12V	37
PD	1163.17	100.48	6.39	9.52	c.35G>T	G12V	27
UTD	1086.48	113.14	UTD	UTD	WT		70
UTD	301.36	241.05	8.82	8.30	WT		74
SD	4414.67	1331.61	3.77	4.67	WT		14
SD	609.57	62.96			c.35G>A	G12D	15
PD					WT		
PD	901.86	459.6	8.30	7.43	WT		68
PD					WT		
PR	3332.21	2042.92	5.17	3.47	WT		6
PD	42.03	78.71	11.81	9.19	WT		48
SD					WT		
PD					c.35G>C	G12A	
PR	1418.75	2411.15	4.91	3.40	WT		4
UTD	872.72	469.76	6.32	5.55	c.35G>A	G12D	71
SD	1384.71	632.61	5.75	5.60	na		9
PD	503.53	206.2	6.83	7.10	na		59

PD	75.64	50.98	10.33	9.52			61
PD	1879.09	587.4	7.50	7.25	na		41
PD	471.68	36.46	5.60	4.77			34
PD	39.27	8.15	12.33	13.18	WT		55
PD	111.94	107.83	10.02	8.30	WT		43
PD					na		
PR					na		
PD	1464.45	298.7	5.94	7.16	WT		51
SD	5533.18	2232.8			na		10
PD	236.8	42.59	8.96	UTD			54
SD	1416.68	819.85			WT		16
PD	719.16	550.72	6.38	5.90	c.35G>A	G12D	42
PD							
UTD	127.95	12.85	9.86	10.64	c.35G>A	G12D	72
PD	331.54	307.55	8.22	6.83	WT		33
PD	936.71	64.49	8.28	10.95	WT		65
PD	132.01	28.72	10.55	12.04	c.35G>A	G12D	35
UTD	760.08	221.16	6.27	8.55			75
PD	162.74	71.16	10.21	11.17	WT		28
UTD	865.02	258.5	7.95	8.94	c.34G>A	G12S	76
PD	489.57	224.81	8.17	7.70	c.35G>T	G12V	46
PD	813.24	529.95	7.16	6.79	c.35G>A	G12D	38
PD							
PD							
PD	1556.84	703.23	5.70	5.40	c.35G>C	G12A	60
SD							
PD	1646.55	1127.43	6.44	5.39	WT		57
PD							
PD	27.71	1.05	13.23	UTD	WT		56
PD	1182.47	76.66	7.48	10.91	c.34G>A	G12S	50
PD							
PD	532.55	171.22	8.87	8.79	c.35G>C	G12A	45
PD	12.43	13.62	UTD	13.67	WT		63
SD	2809.16	804.93	6.13	5.20	WT		17
UTD	1656.76	665.01	6.14	5.07	c.38G>A	G13D	77
SD	18.88	2.2	10.67	12.31	WT		18
SD	1479.28	799.93	5.74	6.28	WT		19
PD	1034.32	384.07	6.64	7.29	WT		53
UTD	24.18	15.47	UTD	UTD	WT		78
UTD	54.13	11.49	9.44	11.32	WT		79
SD	1554.57	646.2	5.23	5.86	WT		20
SD	3536.88	1764.91	5.82	3.45	WT		21
SD					WT		
SD	6390.33	3078.94	3.47	4.02	WT		22
PD							
PD	801.39	486.2	6.81	7.14	WT		40
SD					c.35G>A	G12D	

UTD	1945.99	240.5	8.21	10.16	c.38G>A	G13D	80
PD	1984.72	897.89	4.21	4.31	c.35G>T	G12V	64
SD	5830.27	1980.37	2.58	3.11	WT		23
PD	2321	784.77	5.41	5.21	c.35G>T	G12V	29
PD					WT		
PD	1095.66	468.77	9.03	7.75	c.38G>A	G13D	66
PD	442.29	77.8	9.84	10.39	c.35G>A	G12D	49
SD	1610.75	442.09	5.25	6.21	WT		24
SD	2615.62	1113.89	5.67	7.03	WT		25
PD	1737.75	694.22	6.05	7.01	WT		44
SD					WT		
PD	2271.37	634.05	5.32	5.61	c.35G>A	G12D	39
PD	1858.06	870.14	6.27	6.34	c.35G>A	G12D	52
PD	1018.25	859.41	8.08	5.91	WT		31

There was good correlation between the two methods (for $\log_2$-transformed array data, Pearson > 0.85, $R^2 > 0.7$), with high expression on Affymetrix arrays corresponding to low ΔCt values from TaqMan assays for both amphiregulin and epiregulin (FIG. 9).

Genetic analysis of DNA isolated from tumor biopsies and whole blood:

Somatic mutations in the EGFR tyrosine kinase domain are found to be strongly associated with sensitivity to gefitinib and erlotinib in NSCLC (Janne et al., J. Clin. Oncol., 23, 3227-3234 (2005)). It has been reported that somatic mutations in the EGFR TK domain are not required for response to cetuximab, nor do they appear to be predictive of response to cetuximab (Tsuchihashi et al., N. Engl. J. Med., 353, 208-209 (2005)). Somatic mutations in K-RAS are associated with a lack of sensitivity to gefitinib and erlotinib in NSCLC but their role in cetuximab sensitivity in CRC is unclear (Moroni et al., Lancet Oncol., 6, 279-286 (2005); Pao et al., PLoS Med., 2, e17 (2005)). DNA from 80 tumor biopsies was evaluated for mutations in EGFR, K-RAS and BRAF. Not a single heterozygous mutation was detected in either the EGFR kinase domain or in exon 15 of the BRAF gene. K-RAS exon 2 mutations affecting codon 12 and 13 were detected in 30 out of 80 (38%) analyzed samples (Table 6). K-RAS mutations were detected in only 3 Stable Disease patients out of the 27 Disease Control Group (5 PR and 22 SD) patients tested (11%). On the other hand, K-RAS mutations were detected in 27 out of 53 non-responders (51%). The

data clearly show that the presence of a K-RAS mutation correlates with a lack of response to cetuximab therapy.

Discussion:

The key findings from the analysis of pre-treatment biopsies are that patients whose tumors express high levels of the EGFR ligands epiregulin and amphiregulin are most likely to benefit from cetuximab therapy. In addition, it was found that patients whose tumors do not have K-RAS mutations have a significantly higher disease control rate than those with K-RAS mutations.

The genes for the EGFR ligands epiregulin and amphiregulin are co-localized on chromosome 4q13.3 (Conti et al., Mol. Endocrinol., 20, 715-723 (2006)). It was observed that the expression of epiregulin and amphiregulin was coordinately regulated (Pearson correlation = 0.85). Epiregulin is known to bind more weakly to EGFR and ERBB4 than the EGF ligand, but is a much more potent mitogen than EGF and leads to a prolonged state of receptor activation (Shelly et al., J. Biol. Chem., 273, 10496-10505 (1998)). Elevated expression of epiregulin and/or amphiregulin may play an important role in tumor growth and survival by stimulating an autocrine loop through EGFR. This may characterize a tumor that is "EGFR-dependent" and therefore sensitive to the ability of cetuximab to block ligand-receptor interaction. The observations that constitutive epiregulin and amphiregulin expression in L2987 cells is decreased upon EGFR inhibitor treatment, is stimulated by EGF treatment, and that cetuximab treatment blocks L2987 cell growth, support the hypothesis that these EGFR ligands are beacons of an activated EGFR pathway and perhaps autocrine stimulators. This hypothesis is also supported by results in a lung cancer mouse model in which high expression of epiregulin and amphiregulin, as well as ERBB3, was dependent on EGFR activation (Fujimoto et al., Cancer Res., 65, 11478-11485 (2005)).

It is not surprising that the findings of epiregulin and amphiregulin RNA expression was not translated into protein-based assays. The mRNA transcripts may code for the membrane-anchored precursor forms that are eventually cleaved to generate soluble forms. In the case of amphiregulin, it has been shown that the membrane-anchored isoform, as well as the soluble form, are biologically active and

may induce juxtacrine, autocrine or paracrine signaling (Singh and Harris, Cell Signal, 17, 1183-1193 (2005)). It is interesting to note that in contrast to these findings, elevated serum levels of amphiregulin and TGFα have been reported to predict poor response to gefitinib in patients with advanced NSCLC. (Ishikawa et al., Cancer Res., 65, 9176-9184 (2005)). It remains to be determined whether the tumors of the patients with high serum levels of amphiregulin and TGFα described in that study may have other genetic aberrations such as K-RAS mutation that may allow by-pass of their dependence on EGFR signaling for growth and survival.

Epiregulin and amphiregulin can be used to identify other tumor types that might be sensitive to cetuximab. Epiregulin and amphiregulin expression is increased in androgen-independent prostate cancer cells and after castration in an androgen-sensitive prostate cancer xenograft (Torring et al., Prostate, 64, 1-8 (2005); Torring et al., Anticancer Res., 20, 91-95 (2000)). Epiregulin expression is higher in pancreatic cancer where it stimulates cell growth (Zhu et al., Biochem. Biophys. Res. Commun., 273, 1019-1024 (2000)) and in bladder cancer patients where it is correlated with survival (Thogersen et al., Cancer Res., 61, 6227-6233 (2001)). The enhanced expression of amphiregulin is found to be significantly correlated with overall survival in non-small cell lung cancer (NSCLC) (Fontanini et al., Clin. Cancer Res., 4, 241-249 (1998)). Amphiregulin expression is higher in multiple myeloma cells expressing ERBB receptors and promotes their growth (Mahtouk et al., Oncogene, 24, 3512-3524 (2005)). Recently, it has been found that high levels of lutenizing hormone may elevate the risk of ovarian and breast cancers through the stimulation of epiregulin and amphiregulin which in turn could stimulate mitogenic EGFR signaling (Freimann et al., Biochem. Pharmacol., 68, 989-996 (2004)). Finally, the observation that EGFR and estrogen receptor (ERα) mediate expression of amphiregulin (Britton et al., Breast Cancer Res. Treat., 96, 131-146 (2006)) suggests that a subset of breast cancer patients (EGFR+, ER+, amphiregulin+) may benefit from cetuximab therapy. It is notable that among metastatic breast cancer patients treated with the EGFR inhibitor gefitinib in combination with taxotere, significantly better response rates were seen in ER positive than in ER negative tumors (Ciardiello et al., Br. J. Cancer, 94, 1604-1609 (2006)).

In addition to the observation that the two EGFR ligands are predictive of response to cetuximab, it was found that patients without K-RAS mutations have a higher disease control rate (48%) than those with K-RAS mutations (10%). This result confirms findings from a recently reported study that shows that patients without K-RAS mutations have a higher disease control rate (76%) than those with K-RAS mutations (31%) (Lievre et al., Cancer Res., 66, 3992-3995 (2006)). Interestingly, a majority of the patients described in the previous study were treated with a combination of cetuximab and chemotherapy, suggesting that the K-RAS mutations are predictive of disease progression in both the monotherapy and combination therapy settings. K-RAS plays a crucial role in the RAS/MAPK pathway, which is located downstream of EGFR and other growth factor receptors, and is involved in cell proliferation. The presence of activating mutations in K-RAS might be expected to circumvent the inhibitory activity of cetuximab. K-RAS mutations have also been found to be associated with resistance to gefitinib and erlotinib in NSCLC (Pao et al., PLoS Med., 2, e17 (2005)). These data consistently support the role of K-RAS mutations in predicting response to cetuximab and/or other EGFR inhibitors, and should continue to be evaluated in cancers where RAS mutations are prevalent such as CRC, NSCLC and pancreatic cancer (Minamoto et al., Cancer Detect. Prev., 24, 1-12 (2000)).

In contrast to what has been observed in patients with NSCLC (Janne et al., J. Clin. Oncol., 23, 3227-3234 (2005)), mutations in the EGFR gene (exons 18-21) in the patients enrolled in this CRC study were not detected, confirming the paucity of mutations in patients with CRC (Tsuchihashi et al., N. Engl. J. Med., 353, 208-209 (2005)). Mutations in BRAF (exon 15) were not detected, though such mutations have been observed at a low frequency (<5%) in other studies (Moroni et al., Lancet Oncol., 6, 279-286 (2005)). An increase in EGFR gene copy number was observed in less than 10% of the patients evaluated in this study and while there was a trend towards higher copy number in the patients with disease control, the result was more in line with that of Lievre et al (10% of patients had amplification) than with Moroni et al (31% of patients had amplification). Assessment of the performance of a model using the combination of K-RAS mutation status and epiregulin mRNA expression levels showed excellent prediction power (AUC value of 0.89).

EXAMPLE 3 - PRODUCTION OF ANTIBODIES AGAINST THE BIOMARKERS

Antibodies against the biomarkers can be prepared by a variety of methods. For example, cells expressing a biomarker polypeptide can be administered to an animal to induce the production of sera containing polyclonal antibodies directed to the expressed polypeptides. In one aspect, the biomarker protein is prepared and isolated or otherwise purified to render it substantially free of natural contaminants, using techniques commonly practiced in the art. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity for the expressed and isolated polypeptide.

In one aspect, the antibodies of the invention are monoclonal antibodies (or protein binding fragments thereof). Cells expressing the biomarker polypeptide can be cultured in any suitable tissue culture medium, however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented to contain 10% fetal bovine serum (inactivated at about 56 °C), and supplemented to contain about 10 g/l nonessential amino acids, about 1,00 U/ml penicillin, and about 100 μg/ml streptomycin.

The splenocytes of immunized (and boosted) mice can be extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line can be employed in accordance with the invention, however, it is preferable to employ the parent myeloma cell line (SP2/0), available from the ATCC (Manassas, VA). After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (1981, *Gastroenterology*, 80:225-232). The hybridoma cells obtained through such a selection are then assayed to identify those cell clones that secrete antibodies capable of binding to the polypeptide immunogen, or a portion thereof.

Alternatively, additional antibodies capable of binding to the biomarker polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens and, therefore, it is possible to obtain an antibody that binds to a second antibody. In accordance with this method, protein specific antibodies can be used to immunize an animal, preferably a mouse. The splenocytes of such an immunized animal are then

used to produce hybridoma cells, and the hybridoma cells are screened to identify clones that produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce the formation of further protein-specific antibodies.

EXAMPLE 4 - IMMUNOFLUORESCENCE ASSAYS

The following immunofluorescence protocol may be used, for example, to verify EGFR biomarker protein expression on cells or, for example, to check for the presence of one or more antibodies that bind EGFR biomarkers expressed on the surface of cells. Briefly, Lab-Tek II chamber slides are coated overnight at 4 °C with 10 micrograms/milliliter (μg/ml) of bovine collagen Type II in DPBS containing calcium and magnesium (DPBS++). The slides are then washed twice with cold DPBS++ and seeded with 8000 CHO-CCR5 or CHO pC4 transfected cells in a total volume of 125 μl and incubated at 37 °C in the presence of 95% oxygen / 5% carbon dioxide.

The culture medium is gently removed by aspiration and the adherent cells are washed twice with DPBS++ at ambient temperature. The slides are blocked with DPBS++ containing 0.2% BSA (blocker) at 0-4 °C for one hour. The blocking solution is gently removed by aspiration, and 125 μl of antibody containing solution (an antibody containing solution may be, for example, a hybridoma culture supernatant which is usually used undiluted, or serum/plasma which is usually diluted, e.g., a dilution of about 1/100 dilution). The slides are incubated for 1 hour at 0-4 °C. Antibody solutions are then gently removed by aspiration and the cells are washed five times with 400 μl of ice cold blocking solution. Next, 125 μl of 1 μg/ml rhodamine labeled secondary antibody (e.g., anti-human IgG) in blocker solution is added to the cells. Again, cells are incubated for 1 hour at 0-4 °C.

The secondary antibody solution is then gently removed by aspiration and the cells are washed three times with 400 μl of ice cold blocking solution, and five times with cold DPBS++. The cells are then fixed with 125 μl of 3.7% formaldehyde in DPBS++ for 15 minutes at ambient temperature. Thereafter, the cells are washed five times with 400 μl of DPBS++ at ambient temperature. Finally, the cells are mounted

in 50% aqueous glycerol and viewed in a fluorescence microscope using rhodamine filters.

CLAIMS:

What is claimed is:

1. A method for predicting the likelihood a mammal will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises:

(a) measuring in the mammal the level of at least one biomarker selected from epiregulin and amphiregulin;

(b) exposing a biological sample from said mammal to the EGFR modulator;

(c) following the exposing of step (b), measuring in said biological sample the level of the at least one biomarker,

wherein an increase in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates an increased likelihood that the mammal will respond therapeutically to said method of treating cancer.

2. The method of claim 1 wherein said at least one biomarker comprises epiregulin and amphiregulin.

3. The method of claim 1 wherein said at least one biomarker further comprises at least one additional biomarker selected from Table 1.

4. The method of claim 1 wherein said biological sample is a tissue sample comprising cancer cells and said tissue is fixed, paraffin-embedded, fresh, or frozen.

5. The method of claim 4 that further comprises the step of determining whether said cancer cells have the presence of a mutated K-RAS, wherein detection of a mutated K-RAS indicates a decreased likelihood that that the mammal will respond therapeutically to said method of treating cancer.

6. The method of claim 4 wherein said EGFR modulator is cetuximab and said cancer is colorectal cancer.

7. A method for predicting the likelihood a mammal will respond therapeutically to a method of treating cancer comprising administering an EGFR modulator, wherein the method comprises:

(a) measuring in the mammal the level of at least one biomarker that comprises CD73;

(b) exposing a biological sample from said mammal to the EGFR modulator;

(c) following the exposing of step (b), measuring in said biological sample the level of the at least one biomarker,

wherein an increase in the level of the at least one biomarker measured in step (c) compared to the level of the at least one biomarker measured in step (a) indicates a decreased likelihood that the mammal will respond therapeutically to said method of treating cancer.

8. The method of claim 7 wherein said at least one biomarker further comprises at least one additional biomarker selected from Table 1.

9. The method of claim 8 wherein said biological sample is a tissue sample comprising cancer cells and said tissue is fixed, paraffin-embedded, fresh, or frozen.

10. The method of claim 9 that further comprises the step of determining whether said cancer cells have the presence of a mutated K-RAS, wherein detection of a mutated K-RAS indicates a decreased likelihood that that the mammal will respond therapeutically to said method of treating cancer.

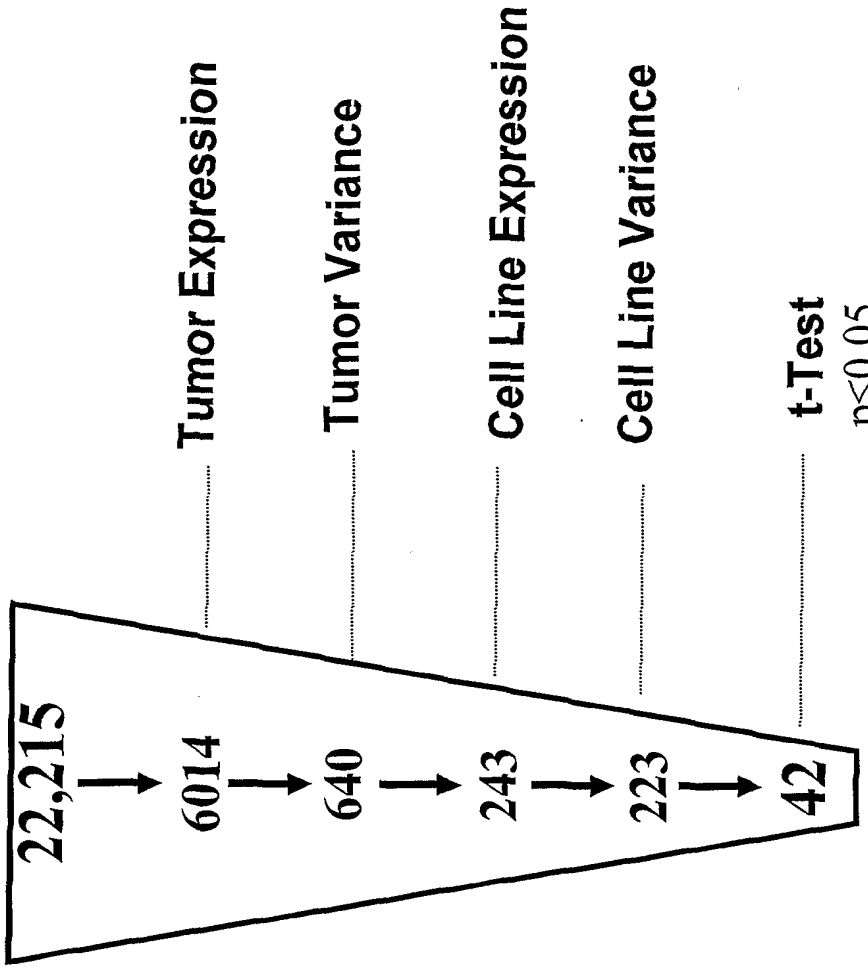
22,215
6014
640
243
223
42
Tumor Expression
Tumor Variance
Cell Line Expression
Cell Line Variance
t-Test
p<0.05

FIG. 1

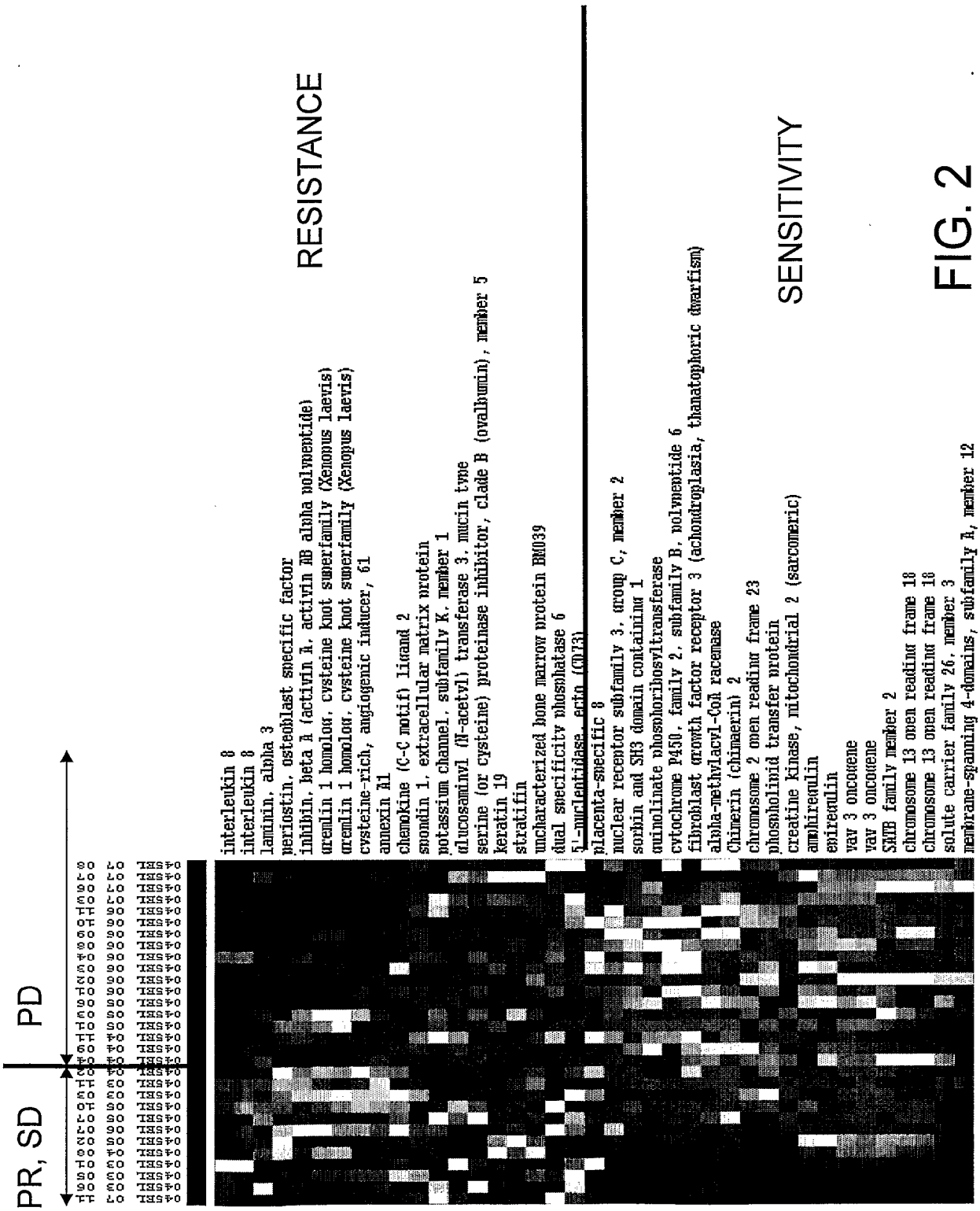
PR, SD
PD
interleukin 8
interleukin 8
laminin, alpha 3
periostin, osteoblast specific factor
inhibin, beta A (activin A, activin AB alpha polypeptide)
gremlin 1 homolog, cysteine knot superfamily (Xenopus laevis)
gremlin 1 homolog, cysteine knot superfamily (Xenopus laevis)
cysteine-rich, angiogenic inducer, 61
annexin A1
chemokine (C-C motif) ligand 2
spondin 1, extracellular matrix protein
potassium channel, subfamily K, member 1
glucosaminyl (N-acetyl) transferase 3, mucin type
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5
keratin 19
stratifin
uncharacterized bone marrow protein BM039
dual specificity phosphatase 6
5'-nucleotidase, ecto (CD73)
placenta-specific 8
nuclear receptor subfamily 3, group C, member 2
sorbin and SH3 domain containing 1
quinolinate phosphoribosyltransferase
cytochrome P450, family 2, subfamily B, polypeptide 6
fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)
alpha-methylacyl-CoA racemase
Chimerin (chimaerin) 2
chromosome 2 open reading frame 23
phospholipid transfer protein
creatine kinase, mitochondrial 2 (sarcomeric)
amphiregulin
epiregulin
vav 3 oncogene
vav 3 oncogene
SAFB family member 2
chromosome 13 open reading frame 18
chromosome 13 open reading frame 18
solute carrier family 26, member 3
membrane-spanning 4-domains, subfamily A, member 12
RESISTANCE
SENSITIVITY

FIG. 2

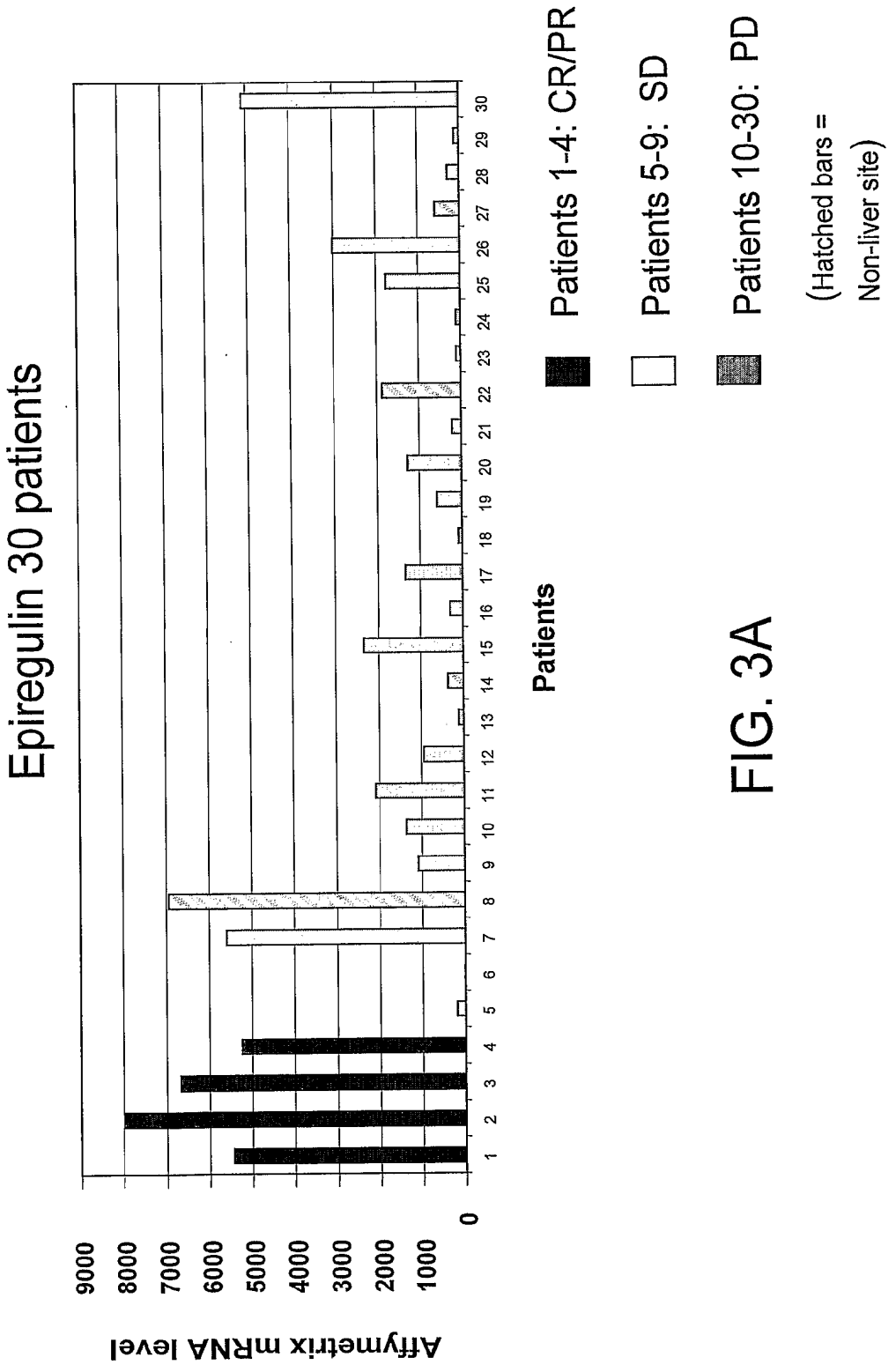
Epiregulin 30 patients
Affymetrix mRNA level
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Patients
Patients 1-4: CR/PR
Patients 5-9: SD
Patients 10-30: PD
(Hatched bars = Non-liver site)

FIG. 3A

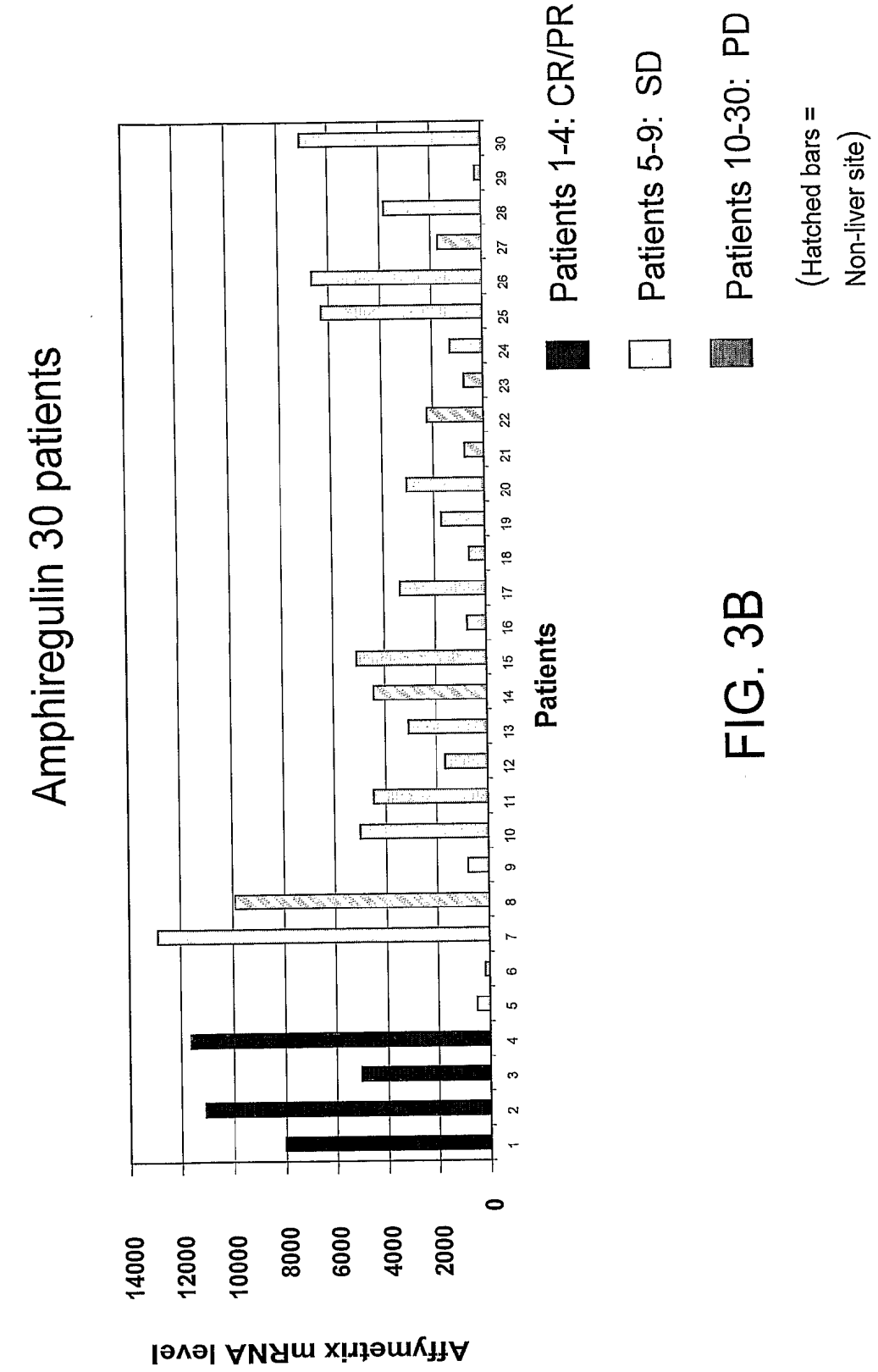
Amphiregulin 30 patients
Affymetrix mRNA level
14000
12000
10000
8000
6000
4000
2000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Patients
Patients 1-4: CR/PR
Patients 5-9: SD
Patients 10-30: PD
(Hatched bars = Non-liver site)

FIG. 3B

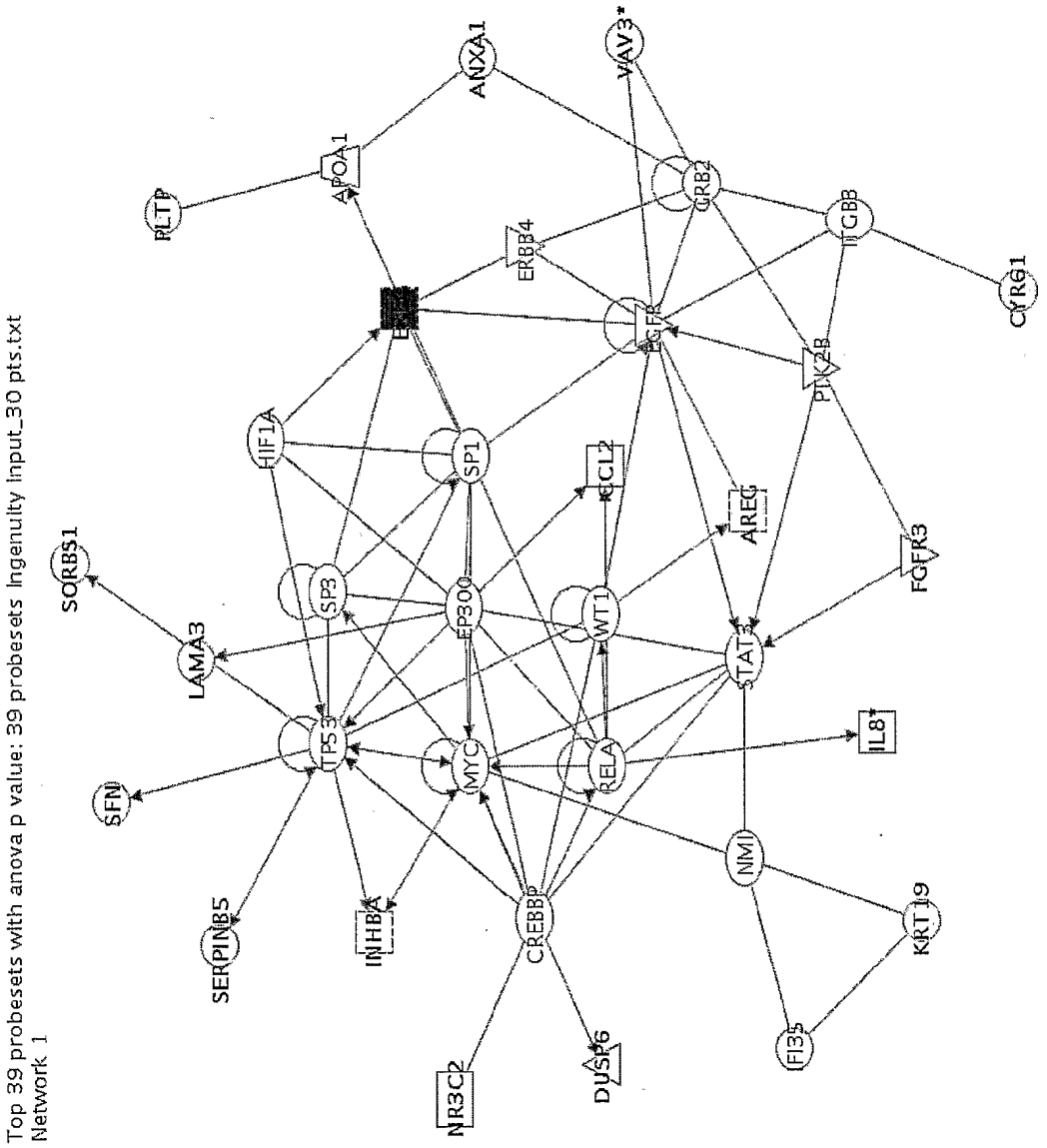
Top 39 probesets with anova p value: 39 probesets Ingenuity Input_30 pts.txt
Network 1
SORBS1
LAMA3
SFN
SERPINB5
INHBA
NR3C2
DUSP6
CREBBP
MYC
TP53
SP3
EP300
SP1
HIF1A
RELA
WT1
CCL2
STAT1
NMI
FI35
KRT19
IL8*
AREG
FGFR3
PTK2B
ERBB4
EGFR
GRB2
IGBB
CYR61
APOA1
RLTP
ANXA1
VAV3*
© 2000-2005, Ingenuity Systems

FIG. 4

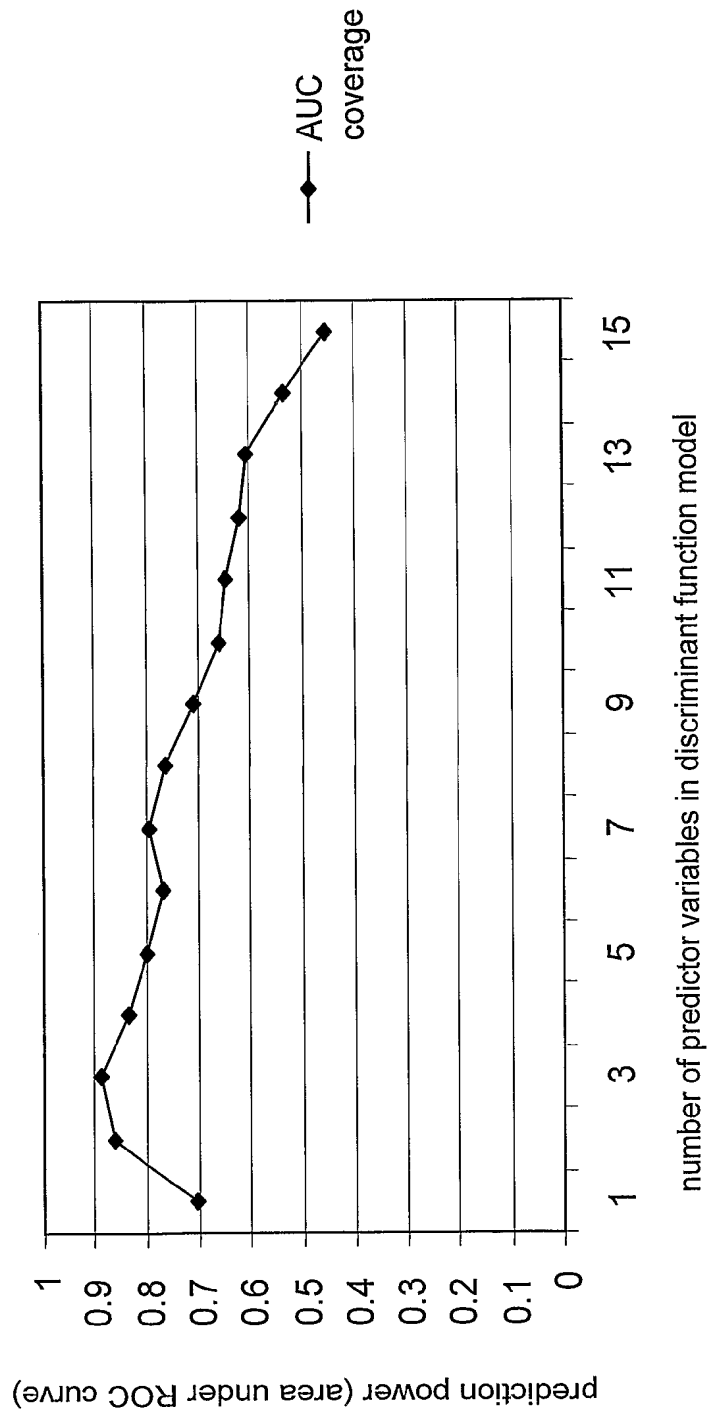
AUC
coverage
prediction power (area under ROC curve)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1
3
5
7
9
11
13
15
number of predictor variables in discriminant function model

FIG. 5

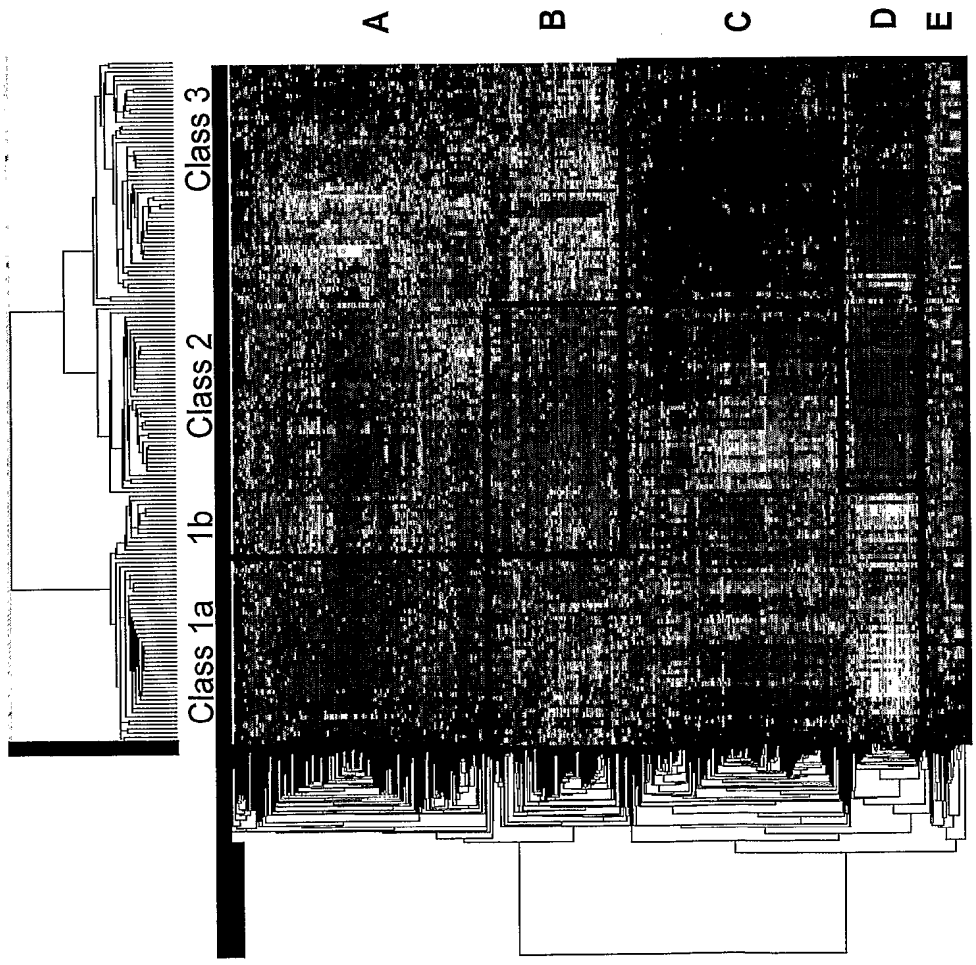

FIG. 6

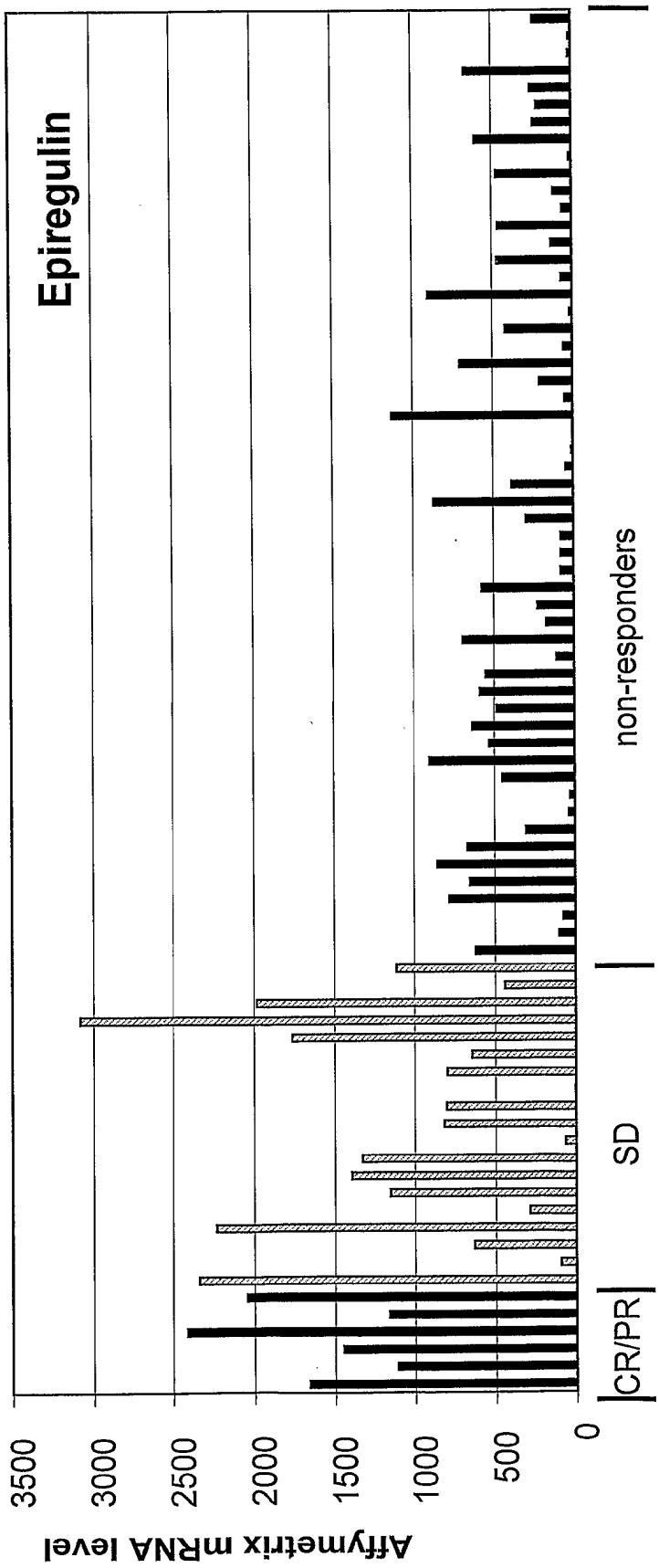
Epiregulin
Affymetrix mRNA level
3500
3000
2500
2000
1500
1000
500
0
CR/PR
SD
non-responders
Subjects

FIG. 7A

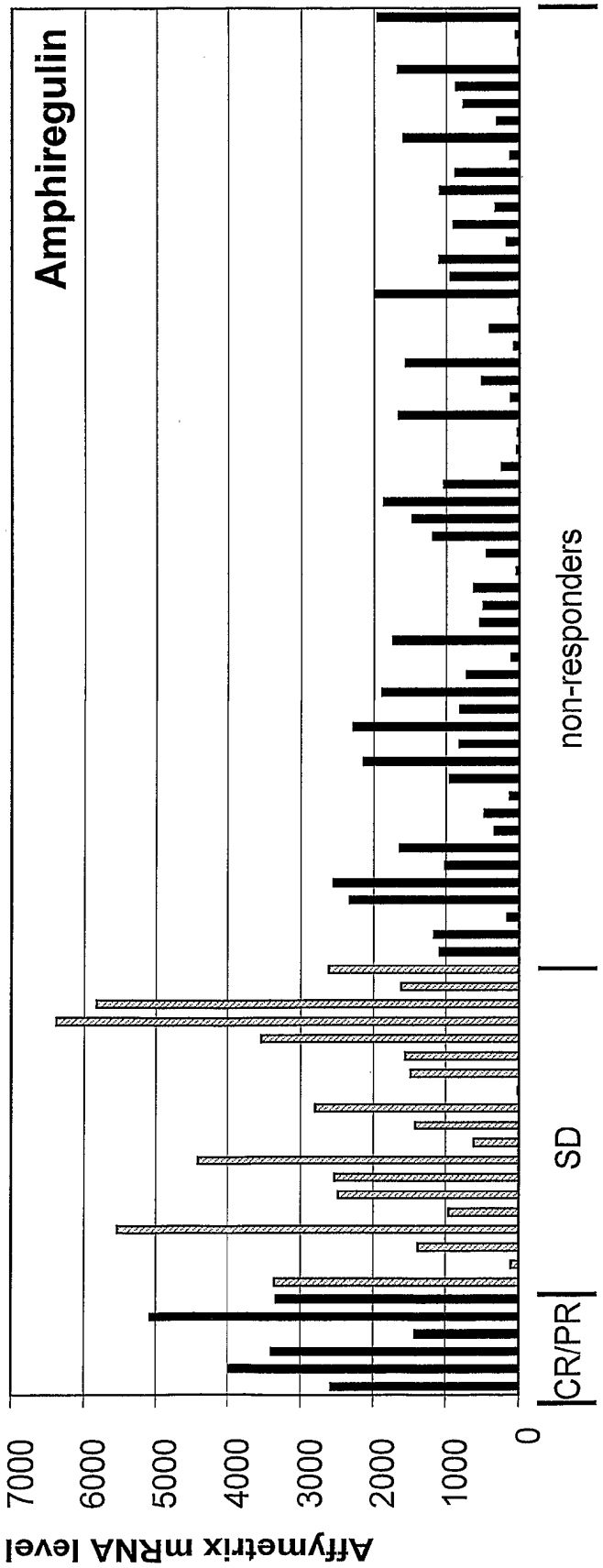
Amphiregulin
Affymetrix mRNA level
7000
6000
5000
4000
3000
2000
1000
0
CR/PR
SD
non-responders
Subjects

FIG. 7B

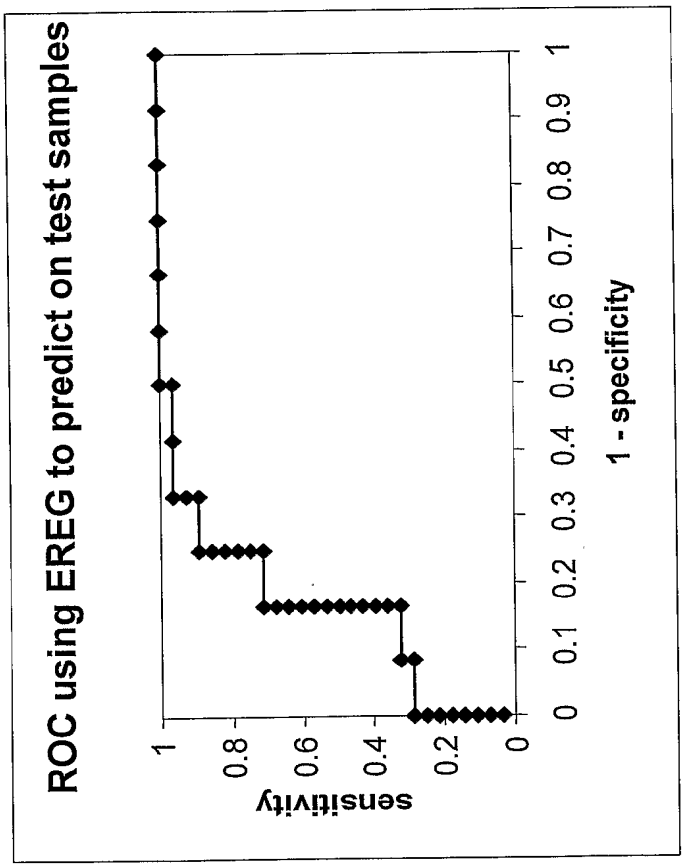
ROC using EREG to predict on test samples
sensitivity
1
0.8
0.6
0.4
0.2
0
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
1 - specificity

FIG. 8A

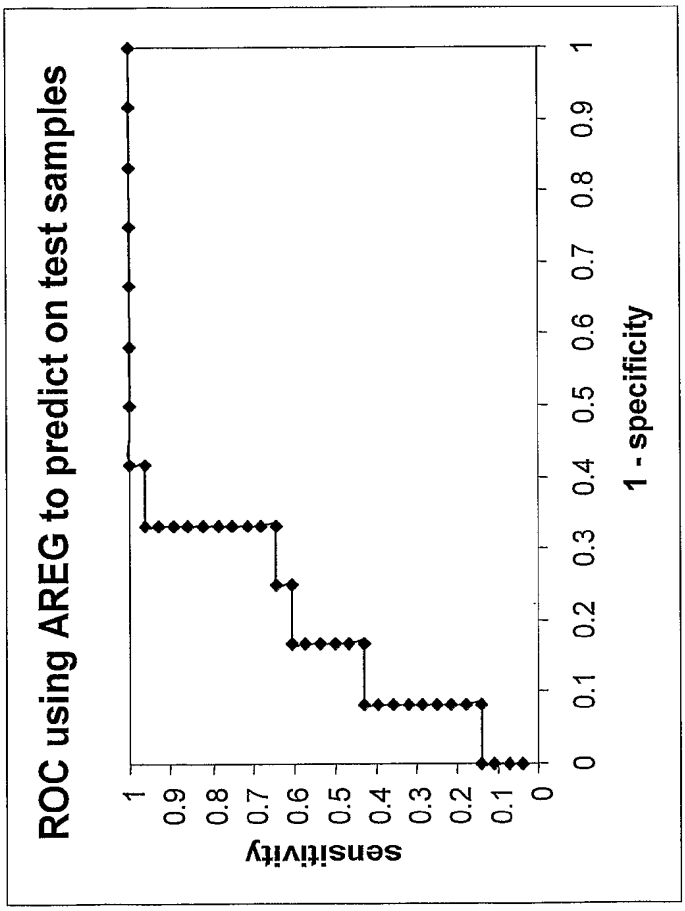
ROC using AREG to predict on test samples
sensitivity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1 - specificity

FIG. 8B

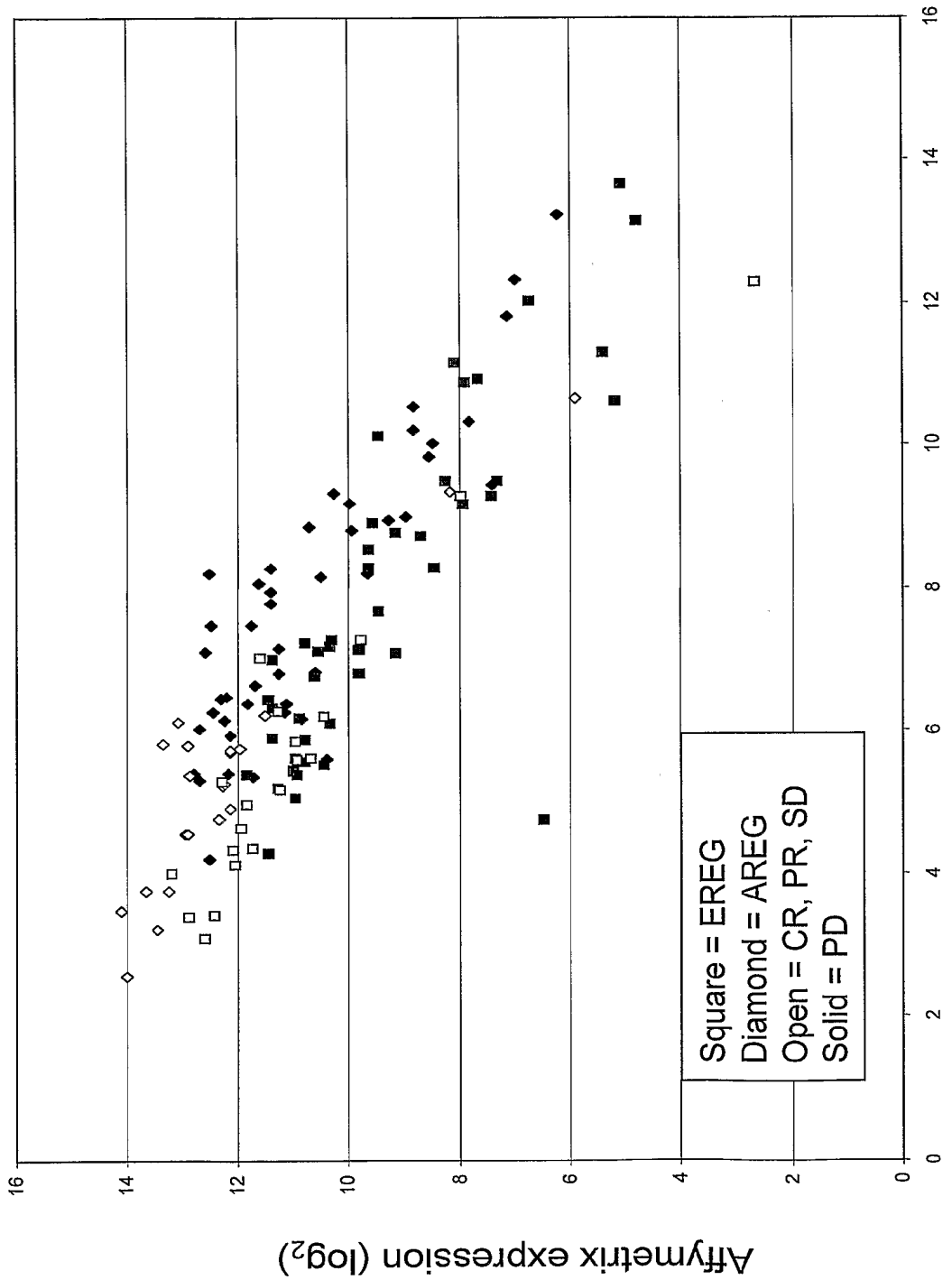
Affymetrix expression (log2)
qPCR delta Ct (dCt)
Square = EREG
Diamond = AREG
Open = CR, PR, SD
Solid = PD
0
2
4
6
8
10
12
14
16

FIG. 9